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INSTRUMENTAL ANALYSIS OF CHEMICAL POLLUTANTS. TRAINING MANUAL

Environmental Protection Agency
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INSTRUMENTAL ANALYSIS OF CHEMICAL POLLUTANTS

CLEAN
WATER

TRAINING MANUAL

WATER QUALITY OFFICE
ENVIRONMENTAL PROTECTION AGENCY

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INSTRUMENTAL ANALYSIS OF CHEMICAL POLLUTANTS

This course is designed for analytical chemists and others having technical responsibility for the collection, identification and measurement of organic and inorganic pollutants in the aquatic environment.

ENVIRONMENTAL PROTECTION AGENCY
Water Quality Office
TRAINING PROGRAM

APRIL 1971

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Water Quality Office, Environmental Protection Agency.

> *Due to the limited availability of the manuals, it is not appropriate to cite them as technical references in bibliographies or other forms of publication.*
>
> *References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Water Quality Office, Environmental Protection Agency.*

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

H M Freeman

H.M. Freeman
Chief, Direct Training Branch
Division of Manpower and Training
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Environmental Protection Agency

TRAINING PROGRAM

Through the Water Quality Office, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

Training is available at five installations of the Agency. These are: the National Training Center located at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio; the Robert S. Kerr Water Research Center, Ada, Oklahoma; the Southeast Water Laboratory, Athens, Georgia; the Pacific Northwest Water Laboratory, Corvallis, Oregon; and the Hudson-Delaware Basins Office, Edison, New Jersey.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and responsibility of the States in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the States is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be obtained through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the States for their own further production and presentation.

An annual Bulletin of Courses is prepared and distributed by the Water Quality Office. The Bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from one of the training centers mentioned above.

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DIVISION OF MANPOWER AND TRAINING

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BEHAVIOR OF SPECIFIC CHEMICAL COMPOUNDS IN THE AQUATIC ENVIRONMENT

I INTRODUCTION

A Discharge of specific chemicals to water may have a variety of effects such as:

1 Oxygen demand

2 Plant nutrition

3 Infection

4 Toxicity

5 Sedimentation

6 Radioactivity

7 Other mineral and organic effects (pH, color, oil, etc.)

8 Heat

This is one classification modified slightly from that used by Eisenhaur[1] which is primarily health-related. Other classifications by effect, by industry, by biological, chemical or physical attributes take a variety of forms.

B A given chemical is likely to be associated with several effects.

1 Oxygen demand may be chemical, biological, or both. Cell mass production, associated with oxidation, results in sediments, plant nutrients, pH effects, etc.

2 Infectious materials commonly are not associated with chemical discharges, but may be as a result of association with infected personnel, pharmaceutical testing, etc.

3 Heated discharges are associated with specific chemicals to the extent governed by process water technology and subsequent treatment. Heat and pressure are common reaction controls in chemical manufacturing or processing.

4 Toxicity is an action induced by too much of any component (exceeding biological tolerance). Toxic agents of common interest are those that exert toxicity at low concentrations.

C Evaluation of Behavior

1 Cursory examination is likely to suggest obvious behavior characteristics on the basis of past experience or short time observation.

2 New or troublesome chemical components may require long-term systematic investigation as outlined[2] to identify unusual behavior that may become associated with major problems in subsequent reuse of the contaminated water.

3 High rate exertion of some behavior aspect is readily apparent. The investigator must guard against low rate effects that do not become apparent except over a long period of time or when gross quantities are involved. There are no "undegradable" materials.

II NATURE OF POSSIBLE CHEMICAL EFFECTS

A Biological effects associated with specific chemicals depend upon whether the chemical may enter the metabolic pathway as a nutrient, interfere with life processes, or coexist with little apparent interaction.

1 Each chemical may stimulate respiration, support it, or become bacteriostatic or germicidal,

depending upon concentration and organism tolerance for that particular chemical and species. Some low concentration commonly stimulates, but each chemical has some concentration at which it is deleterious to metabolism. A pesticide may have all three ranges compressed into the microgram/liter range; sugars may require 20 to 30% concentrations to be inhibitive.

2 A given chemical may be readily available for metabolic reactions or it may react too slowly to be observable except on a long-term basis.

3 Certain chemicals may enter metabolic pathways via common enzyme systems characteristic of many organisms. Others may require selected organisms capable of producing special adaptive enzymes.

4 Certain chemicals may be toxic as a result of destruction of the reversible characteristics of enzyme systems. Malfunction of nerve tissue activity, or interference with some other vial function.

5 A chemical that has little apparent reactability in all mass may thicken cell walls and/or alter transport characteristics across the membrane, may inhibit availability of other essential nutrients by substitution or dilution, and by habitat covering.

B Chemical Effects

1 Acid or alkaline chemicals in excess of the natural buffer capacity of the water affect water quality by making it unsuitable for life processes, irritating, corrosive, or otherwise damaging.

2 Reactive chemicals in water solution may cause formation of precipitates, redissolve boundary deposits, cause formation of undesirable odors, color or taste, and reaction or complexation products of essential materials into a form less desirable or less acceptable for beneficial reuse.

3 Specific chemicals may be manufactured for a specific purpose such as pharmaceuticals, pesticides, growth-controlling hormones, dispersing action, or for stability of response as a protective coating, color, textile, elastomer, container, etc. Mixtures of these and other items in wastewater are not subject to behavior generalities. Behavior is a function of the particular substance and situation.

4 Reactability is a function of the net energy change resulting from the conversion of reactants to products. Those reactions associated with a large net energy release are favored in chemical and biological stabilization. Low net energy yields favor low rate processes.

C Physical Effects

Size, solubility, contact, temperature and pressure largely control favorability among the possible biological or chemical reactions.

1 Aggregate or molecular size controls surface area per unit mass which in turn controls contact possibilities. Large sizes limit reactability. Physical barriers to penetration into the mass or product migration away from the interior are a function of porocity, shape, mass, type and thickness of the material.

2 Rapid reactions are more likely among soluble materials, providing energy release and turbulence are comparable. Equilibrium conditions are shifted toward reaction completion if one or more products are insoluble or otherwise less available.

3 Contact or mixing characteristics determine the probability with which one reacting substance is likely to

enter the reacting zone of another. Air contains abundant oxygen, surface water is likely to approach saturation values for DO. It is rare to encounter adequate oxygen in the vicinity of benthic deposits without vertical mixing.

4 Temperature affects rate of reaction via several mechanisms. A rise of 10° C approximately doubles reaction speed as a result of increased solubility, molecular motion, fluidity, ionization, etc. Increased temperature may have the opposite effect if density stratification decreases mixing or the chemical doesn't follow the trends listed in the preceding sentence.

5 Pressure may promote contact of certain materials but if a gas happens to be a reaction product, increased pressure decreases or changes the course of likely reactions. Increased compaction of sediments is likely to decrease availability for interaction.

6 Changes in physical state form a gas or liquid to solid limits contact to interface zones. Gases of limited water solubility and liquids that are immiscible with water also decrease availability.

III MAJOR EFFECTS OF SELECTED CHEMICALS IN WATER (3, 4, 5)

A Mineral Oil

1 May be discharged in liquid or emulsified form. It is commonly entrapped by cell mass or other particulates to form scum, oily sludge or boundary deposits.

2 Biodegrades slowly at temperatures over 10° C, in concentrations below 10 mg/l under aerobic conditions. Little action anaerobically.

3 Tends to have a deleterious effect upon the biota, produces objectionable tastes, odors, boundary deposits. Covers the bottom with an oily "goop."

B Phenolics

1 Monohydroxy benzene (phenol) is one of the most rapidly biodegraded chemicals known for adapted cultures and concentrations below about 50 mg/l. Noted for oxygen demand.

2 Phenolics tend to produce objectionable odors particularly upon partial chlorination, but may be destroyed by more complete chlorination.

3 Substituted phenolics range from readily degradable to highly toxic or resistant chemicals.

4 Toxic at high concentrations. Tends to break down cell membranes.

C Carbohydrates

This group represents an homologous series ranging from low to high molecular weight components with reactability decreasing as molecular weight increases.

1 The low molecular weight carbohydrates (sugars) are the primary compounds of the food chain and for respiratory energy dynamics. Higher homologues must be hydrolyzed to enter the food chain.

2 Carbohydrates favor rapid cell mass production providing sufficient other nutrients are available to form proteins. Nitrogen and phosphorus are most commonly limiting but other essentials such as growth factors, minerals, etc., may be needed.

3 Prolific slime growth, frequently filamentous, is encouraged by available carbohydrates. Oxygen demand and solids problems are major difficulties.

D Organic Acids

Organic acids, such as acetic, are transitory products of protein hydrolysis and deamination. They may be used in resynthesis or readily oxidized to CO_2 and water. Anaerobic destruction results in CO_2 and CH_4.

1 The discharge of significant organic acids or acid-producing components such as milk commonly leads to low pH problems as a result of the acid release and CO_2 produced from oxidation or rearrangement.

2 Organic acids of low molecular weight are readily available for biochemical transformation with acid reactions produced early, alkaline products in later stages. Feedback of later stage oxidation products favors control of pH and process dynamics.

3 Organic acids such as acetic acid are one step removed from the highest attainable stage of oxidation. They contain a major fraction of total oxygen requirements, hence oxygen demand is a function of the quantity of material rather than oxygen requirements per mole. Stabilization favors oxidation and rearrangement to a greater extent than resynthesis.

4 Higher homologues of the series show decreasing availability and pH effects.

E Amines

Amines are a common product of proteolysis (protein hydrolysis); deamination results in formation of organic acids and ammonia. The amino acid series includes many items essential to protein and enzyme synthesis. Mixed organisms are likely to be capable of synthesizing necessary amines from the mineral components but one specie is unlikely to have the capacity to synthesize all of the required amino acids. Availability from preformed sources decreases the need for synthesis.

1 Amine compounds are among those contributing objectionable odor characteristics when present in significant amounts in water (putrid odors).

2 Stabilization dynamics favor deamination of excessive amines to produce acids and ammonia. Carbon hydrogen and nitrogen oxygen demand may be very high in degrading fractions while solids problems and oxygen demand are characteristic of growing systems.

3 High reactability of primary amines encourages high rate conversions. Secondary and tertiary amines show decreased reactability.

F Pesticides take a variety of forms, all of which are intended to disrupt metabolic processes. They may be highly persistent such as DDT and Chlordane, or readily hydrolyzed such as certain phosphorus and carbamate products. Distribution and persistence are major factors pertinent to behavior in water.

1 Organic pesticides are likely to be more soluble in oily materials than in water. They tend to become distributed accordingly. It is common to find very high concentrations of the pesticide in or on cell mass, organic residues, oily materials or surface films, or on other active surfaces such as silt.

2 Pesticides seem to be selective in the degree of effect among organisms. Slime organisms tend to concentrate organic pesticides to a high degree but have a high tolerance factor. Certain organisms such as fish are highly sensitive and tend to be the victims of pesticide concentration through the food chain as well as by other contact.

3 Pesticide concentrates in bed loads, boundary deposits, living organisms and ducts tend to be transported widely by motility, air or water carriage unless the compound is subject to natural degradation to innocuous products.

G Detergents - Surfactants

A detergent is a cleaning agent consisting of a surfactant (surface active agent) and various items added in process, builders to aid detergency, and special additions added for sales appeal, shelf storage, or usability.

1 Detergents are unique among manufactured products -- essentially all of that used is discharged in the carriage water along with the removed contaminants of the cleansed items.

2 Surfactants are intended to reduce surface tension. Cell membranes are characterized by certain surface active properties which may be altered by surfactants.

3 Most treatment operations tend to promote agglomeration and/or oxidation. A detergent generally contains dispersing agents which complicate treatment technology.

4 Detergent production has been modified to improve treatability. Treatment plant capacity and control need upgrading in line with the load and situation.

H Coloring Agents - Dyes

Organic coloring agents include many complex agents designed to produce a color effect that is resistant to changes induced by light, heat, chemicals and time. The variety of chemicals used causes shifts in behavior and applicable means of treatment.

1 Color is the primary problem. Its removal usually requires physical-chemical treatment because dyes are likely to be inhibitive to organisms, and are not readily removed in process. Storage tends to equalize colors and blend them into a conglomerate amber hue with some removal in process.

2 Acid or alkaline agents are commonly used to "fix" the colors. It is unlikely that coloring operations will result in acid/base equivalency without additional neutralization.

I Inorganic Salts

Chlorides or sulfates may be added to water as a result of domestic use water treatment, manufacturing, mining, or many other operations.

1 Sodium potassium or other monovalent cations tend to produce soluble salts that may interfere with beneficial uses of water as a result of taste, osmotic pressure changes, cathartic action or other effects. Soluble mineral salts commonly are inefficiently removed during conventional treatment. Distillation electrophoresis reverse osmosis, freeze-out, and certain other procedures are more feasible.

2 Certain multivalent action sulfates are relatively insoluble and may be precipitated. Chlorides generally are quite soluble. The cation may be converted to insoluble carbonates, but any substitution reaction is likely to produce soluble components similar to those in I-1 that limits total dissolved solids removal.

3 Deposited material frequently may be redissolved under favorable conditions. That which remains tends to fill the deposit area and make it less desirable for aquatic growth or beneficial reuse.

REFERENCES

1 Eisenhaur, Dr. H.R. Water Pollution and Health. Water & Pollution Control (Canada) 107:24. March 1969.

2 Ludzack, F.J., and Ettinger, M.B. Estimating Biodegradability and Treatability of Organic Water Pollutants. Bio. Tech. & Bio. Eng. V:309. December 1963.

3 Ludzack, F.J., and Ettinger, M.B. Chemical Structures Resistant to Aerobic Biochemical Stabilization. Jour. Water Pollution Control Federation 32 (11):1173. November 1960.

4 Information Service on Toxicity and Biodegradability (INSTAB) Ministry of Technology, Water Pollution Research Laboratory, Stevenage, Herts, Great Britain. Dr. Al Downing, Director. (1966 + 2 supplementary lists, 1967 and January 1969)

5 Behavior of Organic Chemicals in the Aqueous Environment. Wash. Univ. Part I: Literature Critique 1966 ($4.00). Part II: Behavior in Dilute Solutions 1966 ($3.00).
1825 Connecticut Avenue, N.W.,
Washington, DC 20009.

This outline was prepared by F.J. Ludzack, Chemist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

INSTRUMENTATION IN WATER CHEMISTRY

I CHANGES IN WATER AND WASTE CHEMISTRY

A Water and Sewage Treatment Plants

1 In the past 40 years, treatment plants have increased in complexity and efficiency, utilizing new processes to accomplish a higher degree of treatment, in order to meet the need for expanded water use and to reduce water pollution.

2 Because of the sophisticated systems now in use, and the need for still further improvement in treatment, the associated analytical work to monitor the treatment processes and to evaluate the quality of the end product has become more difficult.

B Industrial Waste Analyses

1 Rapid industrial growth has greatly increased the problems of waste effluent characterization and the monitoring of the effects on receiving waters. Analytical procedures are now required for compounds which did not exist a few years ago.

2 The large increase in sources of industrial wastes has produced a significant increase in the workload of the average pollution control laboratory.

C Increased Analytical Requirements

1 The modern laboratory must utilize analytical procedures for a large number of specific pollutants, present in extremely small concentrations. For example, the 1936 edition of Standard Methods of Water Analysis lists approximately 40 determinations for water and wastes, while the 1965 edition includes more than 30 additional measurements which can be made.

Toxicity studies are continually indicating the need to measure pollutants in the submicrogram range, because of subtle, long-term effects on aquatic and human populations.

2 To meet the current needs, laboratory instrumentation must be utilized wherever possible. In many cases, the required specificity, speed, and reliability cannot be accomplished by the older manual procedures. The use of automated equipment effectively reduces manpower requirements.

II ADVANTAGES AND LIMITATIONS OF INSTRUMENTS

A Advantages

1 Speed

Many instrumental methods are quite rapid when compared to traditional bench methods. For example, metals by atomic absorption can be determined at the rate of 30-50 per hour, while the corresponding colorimetric methods are very tedious and time-consuming. Autoanalyzer techniques permit 20-40 determinations per hour, as contrasted to 5-10 per hour by older methods. Separation techniques by gas chromatograph are extremely rapid.

2 Reduced error

When properly calibrated and maintained, instruments are much less subject to random manipulation error. The Autoanalyzer precision is markedly superior to even the best manual techniques, due to mechanical reporduction of sample measurement and reagent addition.

3 Data handling

Modern laboratory instruments often provide direct concentration readout, eliminating calculation. Automatic printing of final results is available for many instruments.

4 Automation

Instruments can often be adapted to incorporate automatic sampling, analysis, and final recording. When coupled to computers, the entire process through data storage can be automated.

B Limitations

1 Availability

Dependable instrumentation, at reasonable costs, is not available for many analyses performed in the water laboratory. For example, satisfactory instrument packages are not available for solids, biochemical oxygen demand, color, chemical oxygen demand, cyanides, phenolics, sulfide, or threshold odor.

2 Sample variation

Instruments cannot provide judgment. They cannot recognize or compensate for extreme changes in concentration, interfering substances, precipitates, or lack of proper sample preparation.

3 Lack of control

Most instruments are not equipped to indicate that the system is not working properly or that mechanical or electronic failures are producing erroneous results.

III DESIRABLE DESIGN CHARACTERISTICS

A Specificity and Adaptability

The instrument should actually measure the constituent or sample characteristic and nothing else. However, provision should be made to adapt the equipment to measure a number of similar elements or compounds.

B Rapidity

To utilize the advantages of speed, the instrument should be capable of performing complete analyses in a relatively short time, generally a few minutes.

C Ruggedness

The equipment should be able to maintain proper operation without frequent readjustment or recalibration and should withstand daily usage under normal laboratory conditions. Excess maintenance or frequent breakdown reduces the value of laboratory instruments.

D Data Processing

Ideally, the design should incorporate automatic calculation and final printout of the data in the appropriate concentration units.

IV QUALITY CONTROL OF INSTRUMENTAL ANALYSES

A Instrument Design

The most important factor in assuring reliable data in instrumental analyses is the design of the equipment. Within any class of laboratory instruments, design varies from very poor to excellent. In addition to basically similar circuitry or optics, some instruments provide better control of drift, simplified calibration or adjustment, superior readout, or useful accessories. The desirable features above should be considered in selecting instruments.

B Standardization

In the performance of instrumental measurements, calibration by means of known reference values is very important. The analyst should establish a routine calibration system and should determine by experimentation the required frequency of recalibration. In the case of strip-chart recording, digital presentation, or automatic printout, the entire system, including final data presentation, should be checked and calibrated.

C Controls

It is a serious mistake to assume that because an instrument was working properly it will continue to do so. The method used should include a sufficient number of standards, spiked samples, or other controls to establish satisfactory performance during routine operation. Since the instrument cannot distinguish a false value from a true one, the analyst must provide the needed control.

V NEEDS AND FUTURE DEVELOPMENTS

A Specific-Ion Electrodes

The recent development of specific-ion electrodes has provided a new analytical tool for the water and waste chemist. Research efforts to improve existing systems and to develop new electrodes should expand the use of this instrumental approach.

B Gas Chromatograph Detectors

New detector systems are needed to expand the usefulness of gas chromatography for organic pollutant measurements. Improved detectors for nitrogen, phosphorus, and hydrocarbons would increase the ability to detect and measure organic compounds containing these elements and structures.

C Automation

The current trend toward complete automation of laboratory tests will be beneficial to the analyst. While providing more rapid, repetitive determinations, increased automation will require greater attention to quality control procedures.

D Data Handling and Computer Interfacing

Many instruments are now being designed to provide digital concentration readout and machine printing. The next logical step will be direct connection to a small computer in the laboratory or suitable interfacing to a time-sharing facility. Such systems are now available for complex instruments such as mass spectrographs and NMR and will in the future be available for even the simplest laboratory instruments. For example, a computer system has been developed to continuously accept the signals from up to 30 gas chromatographs simultaneously and to provide printouts of the values for each instrument.

This outline was prepared D.G. Ballinger, Director, Analytical Quality Control Laboratory, Division of Research, WQO, 1014 Broadway, Cincinnati, OH 45202.

AUTOMATION OF CHEMICAL ANALYSIS

I NEED FOR AUTOMATED ANALYSIS

A At least 30 samples per week for any particular analysis.

B The more complex and manipulating time required for manual method the greater the advantages to automate.

C Simple manual methods requiring one titration or instrument reading may be faster manually unless there are more than 100 samples per work day.

II DEGREE OF AUTOMATION

A Maximum automation - direct from sampling source to print-out of results of multiple analysis.

B Medium automation - individual samples, instrumental analysis, recording and calculating data.

C Minimum automation - simplicity and efficiency in manual analysis.

III PRESERVATION OF SAMPLES

A Biological and Chemical Stability

  1 Store at 5°C, quick freeze

  2 Low pH

  3 Chloroform 1%

  4 Mercuric chloride 100 mg/l

  5 Formalin 500 mg/l

  6 Potassium cyanide 50 mg/l

  7 Thymol 250 mg/l

IV AUTOMATED INSTRUMENTATION

A Sensory

  1 Titrimetric

  2 Potentiometric

  3 Photometry - flow through cells

    a Visible range

    b Ultra violet range

    c Flame

  4 Polarographic - DO

  5 Others as coulometric, conductometric, etc.

V MANUAL SYSTEM WITH INSTRUMENTS

A Automatic pipettes and burettes and reagent dispensers

B Electrical on and off timers

C Glassware, test tubes vs. volumetric flasks

D Small volumes

E Combining reagents

VI CONTINUOUS FLOW SYSTEMS

A Pumping Systems

  1 Peristalic

  2 Piston

  3 Vibrator

B Reagent Addition

C Removal of Interferences

  1 Filtration

  2 Dialysis

- 3 Distillation
- 4 Chemical complexation
- 5 Extraction

D System Hydraulics

- 1 Tubing and glassware
- 2 Mixing
- 3 Temperature control
- 4 Time control
- 5 Digestion

VI RECORDERS

A Meters

B Chart paper, single pen and multi pens

C Print-out data

VII TECHNICON CORP. - AUTO-ANALYZER

A Equipment and Principle

B Adaption of Standard Methods to Auto-analyzer

- 1 Sensitivity
- 2 Wash-out
- 3 Chemical reaction and control

C Methods currently in use

- 1 ABS
- 2 Ammonia nitrogen
- 3 Nitrite and nitrate nitrogen
- 4 Phosphate

D Advantages of Auto-Analyzer

- 1 Speed - automatic clocks
- 2 Small volume of sample required
- 3 Removal of human error
  - a Timing reactions
  - b Reading spectrophotometers
  - c Error in pipetting, recording, etc.
- 4 Permanent record of data
- 5 Ease of checking every 10 or 20 samples against standards
- 6 All samples treated exactly the same
- 7 Use of chemical methods which cannot be used manually.

E Disadvantages

- 1 Acceptable manifold
- 2 Loss of accuracy in determination of a low concentration when proceeded by a high concentration (50 fold)
- 3 Wash-out difficulty
- 4 Mechanical failure
- 5 Pump tubes

F Gradient dilution technique

G Influent and effluent pumped at same rate

$$C_t = C_1 (1 - e^{\frac{-rt}{v}})$$

C_1 = conc. of influent

C_t = conc. effluent at t time

t = time (min.)

r = pumping rate (ml/min)

v = volume in flask

H Simultaneous Analysis of Nitrite and Nitrate

1 History and chemistry

2 Manifold

3 Variables investigated

4 Accuracy and precision

REFERENCES

1 Muller, R.H. Fully Automatic Titrator Has Varied Applications. Anal. Chem. 29, 61 A. 1957

2 Patterson, G.D., Jr. Automatic Operations in Analytical Chemistry. Anal. Chem. 29.605. 1957

3 Ibid. Anal. Chem. 31. 646. 1959

4 Ryland, A.L., Pickhardt, W.P., and Lewis, C.D. Automation Techniques in Analytical Chemistry. E.I. duPont de Nemours & Co. Wilmington, Del.

5 Kamphake, L.J. Simultaneous Analysis of Nitrite and Nitrate in Water and Sewage. In Press, R.A.T. Sanitary Engineering Center. Cincinnati, Ohio.

6 Automatic Chemical Analysis. Annals of the New York Academy of Science. Vol. 87. Art. 2. 1960.

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TODAY'S APPROACHES TO THE IDENTIFICATION AND MEASUREMENT OF ORGANIC CHEMICALS IN WATER

I INTRODUCTION

The term identify as used in chemical analysis means the assignment of a molecular or ionic structure to a chemical substance so that it might be distinguished as a single substance, never changing and different from other chemical materials. When attempting to identify and quantitate organic chemicals in water, it is necessary to be familiar with some of the techniques used in the identification of organic compounds in general. Therefore, the emphasis of this outline will be placed on those methods used in the general organic analysis with emphasis placed on methods that are applicable to water analysis.

II DETERMINATION OF PHYSICAL CONSTANTS IN ORGANIC ANALYSIS

A Melting Points

B Boiling Points

C Refractive Indices

D Density

E Optical Rotation

F Molecular Weights

All of the above methods are used on relatively pure organic compounds but their usefulness in water would come after separation and cleanup procedures. For example, the refractive index, which measures ratio of the velocity of light in a vacuum to that in the substance, can be used in the examination of fractions from distillations. The refractive index is invaluable in the eliminating of isomers.

III PRELIMINARY STEPS IN THE ANALYSIS OF ORGANIC SUBSTANCES

A Elemental Analysis - Although it is assumed for the purposes of this text that the materials being examined are organic compounds, several elements are found in connection with those materials.

1 Sodium fusion - the easiest method for determining sulfur, nitrogen and halogens is to convert them to ions. Nitrogen is converted to cyanide ions, sulfur to sulfide ions and halogens to halide ions. The ions then can be identified by conventional methods.

IV CLASSIFICATION BY SOLUBILITY

Solubility depends on the interionic or intermolecular forces exhibited by both the solute and the solvent. In order for one substance to become dissolved in another, bonds must be broken, and new bonds formed between the molecules or ions of both the solute and solvent.

Since ionic substances do not dissolve in water unless they can form hydrogen bonds with water molecules, many organic compounds will not be found to dissolve in water.

By using the carbon-chloroform extract and applying the techniques of solubility separation to it, organic groups and classes can be isolated.

V PHYSICAL METHODS FOR THE DETERMINATION OF FUNCTIONAL GROUPS IN ORGANIC COMPOUNDS

A Chromatographic Procedures - Chromatography is applied to separation or fractionation procedures based on the process of differential migration. The sample is in a mobile phase, either liquid or gas, and is passed through a column which contains a stationary phase on which the mixture is adsorbed. The stationary phase is a solid, or a liquid held immobile by a solid.

1 Paper chromatography - paper serves as the support.

2 Column chromatography - the immobile adsorbent phase is a solid and contained in a glass column.

3 Thin layer chromatography - using thin layers of adsorbents on glass plates. Thin layer is popular because of the low cost of equipment, simple methods, speed, good separations, and the sensitivity of detection of the fractions. It also should be pointed out that all chromatographic principles taking place in solid-liquid and liquid-liquid systems can be used. Adsorbtion chromatography is the most commonly used method, but ion exchange, partition, and reversed-phase partition can be applied in TLC.

TLC has many applications in water chemistry. Its sensitivity makes it possible to detect trace amounts of pollutants such as pesticides. Another use of TLC is using the technique in conjunction with other methods of analysis. For example, as a cleanup and separation for Infrared Analysis.

4 Gas chromatography - this technique requires that a solute undergo distribution between two phases, one the stationary phase and the other a moving or mobile phase. It is the mobile phase that carries the solute through the column until it emerges from the end of the column separated from other solutes. The detection system can be electron capture, flame ionization, thermal conductivity, microcoulometric, Coulson electrolytic conductivity detector, or flame photometric detector. As in most fields, the gas chromatograph has found wide application in the water pollution control field. It has been the basis of pesticide and other types of organic separation and identification such as phenols and natural products.

B Infrared Spectrometry - It has been found that nearly all chemical compounds showed marked selective absorption in the infrared regions. Infrared spectra are for the most part very complex. The greatest use for IR is to detect functional groups that are most commonly encountered in organic compounds. When light strikes a substance, a portion of the incident light is reflected, another transmitted, and a portion is absorbed by the molecules of the substance. The absorption of the infrared radiation displaces the atoms from their normal positions and causes them to oscillate. In order to measure the particular wavelengths which a homogenous system of organic molecules will absorb, it is necessary to use a beam of infrared light, and allow it to fall on a sample of the molecular species under investigation. Then we can measure which frequencies or wavelengths were absorbed by determining from the transmitted light which frequencies are missing.

One result of the complexity of infrared spectra is that it becomes highly improbable that any two different compounds will have identical curves. Therefore, an infrared spectrum of a pure compound presents a definite method of identification.

Infrared analysis after separation and possible identification by gas chromatography has found extensive use in water analysis.

C Visible Spectrometry - This involves the determination of the light absorbing capacity of a compound. The region of the electromagnetic spectrum which visible light encompasses is approximately 380 to 750 mμ. Since it is colored systems that exhibit selective light absorptive capacity, the term colorimetric analysis is often used when the objective is to determine the concentration of the chemical responsible for the color. The actual system does not involve either the nature of the colorant or its amount. In chemistry, a better term is absorptimetry.

The main problems in methodology of visible spectrometry are:

1 To make a suitable colored solution.

2 To measure the light absorptive capacity of this solution, or to compare it with that of a colored solution of known concentration.

Sometimes compounds are naturally colored but usually it is necessary to develop a color by the addition of one or more color-forming reagents. Visible spectroscopy plays a large part in the instrumentation of organic analysis in water. For example, the ammonia series is analyzed by spectrophotometers as are phenols.

D Ultraviolet Spectrometry - The use of near ultraviolet absorption spectra as an analytical tool has been greatly increased as recording ultraviolet spectrophotometers have become available.

Selective absorption among organic chemicals is related to a deficiency of electrons in the molecules. Completely saturated compounds show no selective absorption throughout the visible and ultraviolet regions. Compounds which contain a double bond absorb strongly in the far ultraviolet. Conjugated double bonds produce absorption at longer wavelengths.

The molar absorptivity depends on the rate at which the absorber is absorbing radiant energy from the beam of incident light.

Since the solutions exhibiting ultraviolet absorptivity may be colorless, the concentration of the absorber by visual examination is impossible.

Ultraviolet spectroscopy has been applied to many phases of organic analysis in water, the identification of aromatic hydrocarbons and conjugated aliphatics. In biochemical work, ultraviolet spectra has often been used to identify degradation products.

Often times information about an unknown structure can be obtained by a direct comparison of its absorption spectrum with those of known structures.

E Mass Spectrometry - The mass spectrometer sorts out charged gas molecules (ions) according to their masses. There are four methods of producing mass spectra. In all methods, ions are produced by collisions of rapidly moving electrons with the gas molecules to be examined. The electrons ionize the neutral gas molecules by knocking out one or more of the orbital electrons. The resulting positive electrons are then removed from the gas stream as fast as they are formed by using a negatively charged accelerating electrode. The remaining gas molecules which did not become ionized are removed by a vacuum pump.

In review, certain aspects of mass spectrometry are fundamental. First, if the sample is organic, it must have an obtainable vapor pressure of at least 10^{-7}mm Hg. The higher the vapor pressure, the easier the mass spectrum is obtained. Second, ions must be prepared from the sample molecules. Third, positive ions are mass analyzed. The fourth is the mass spectrum. The mass spectrum is recorded on a strip chart or on photosensitive paper.

The mass spectrometer has the following applications: Determination of nominal molecular weight. The procedure is to measure the mass at which the ion corresponding to the molecule occurs. Normally the method is accurate to the whole number. Instruments with sufficiently high resolving power to permit the exact formula of a molecule to be computed from a single measurement of the exact mass of the ion are available.

In any total structural determination, the mass spectrometer will usually play an all important part. Since the complete mass spectrum is so complex, it is not possible to determine the exact structure of any molecule of molecular weight of about 150 or greater with the mass spectrometer alone. The use of infrared spectroscopy and nuclear magnetic resonance spectroscopy in conjunction with the mass spectrometer is absolutely necessary.

The combination of a fast scanning mass spectrometer with gas chromatography has found extensive use in characterization.

The methods and tools used in the identification and measurement of organic chemicals are numerous and in many cases complicated. The uses of the various techniques and instrumentation as related to water chemistry is much too broad to cover in a paper of this nature, but an introduction to some of the possibilities of their uses should be gained.

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TRACE ORGANIC CONTAMINANTS IN WATER

I INTRODUCTION

The subject of trace organic contaminants in water continues to receive increasing amounts of attention. Concurrently, the sources of these refractory materials are becoming more varied and complex. The problems associated with these substances are likewise increasing and satisfactory water treatment is becoming more and more difficult.

II SOURCES

A Man Made

1 Domestic wastes - In various stages of sewage treatment, discharged into rivers and streams.

2 Many complex industrial wastes, which have been accidentally or deliberately spilled or dumped.

3 Carrier solvents, such as those used in pesticide formulations.

4 Chemicals, such as fertilizers, which are applied directly to the land and water.

5 Petrochemicals.

B Natural

1 Extracellular products of algae by

a Diffusion of metabolic intermediates;

b By-products of metabolism;

c Hydrolysis of capsular materials.

2 Actinomycetes - Micro-organisms present in rivers/streams by their growth and decomposition cycles.

3 Bacterial decomposition of organic materials.

4 Run-off of land vegetation and soil.

5 Sediments, through decomposition and subsequent exchanges.

III CONCENTRATIONS

A Waste outfalls contain from a few ppm up to 13% of organic contaminants.

B Surface waters contain organic contaminants in concentrations ranging from less than ppb to several ppm.

C Organic refractory substances cause serious problems in comparative trace amounts.

IV PROBLEMS

A Taste and odor are usually the first effects noticed.

B Increased chlorine and carbon demand interfere with coagulation and increase treatment costs.

C Fouling of ion exchange resins and malodors in food and beverage industries.

D Adverse effects on aquatic forms that support higher aquatic life, off-flavors in fish flesh, and direct toxic effects on fish.

E Potentially long-term toxic effects and possible carcinogenic effects on humans.

V COLLECTION, ISOLATION AND IDENTIFICATION TECHNIQUES

A Sample Collection

1 Grab samples from 1 to 5 liters are adequate in many cases.

2 The carbon adsorption method (CAM) is the most common concentration technique.

3 The megasampler, a scaled-up version of the CAM, is used in special studies where large quantities of organic materials are needed.

B Isolation

1 The carbon samples are sequentially extracted with chloroform and ethyl alcohol to desorb the organic material from the carbon.

After extraction, the excess solvent is removed, and the samples are brought to dryness to yield:

a) Carbon chloroform extract (CCE).

b) Carbon alcohol extract (CAE).

2 The CCE is separated into broad classical groups by techniques based on solubility differences.

3 Further separations are made by such techniques as adsorption, paper, and thin layer chromatography.

C Identification

1 Tentative identification is normally made using gas chromatography, paper chromatography, thin layer chromatography, and fluorescent spectroscopy.

2 Positive identification generally requires the use of infrared spectroscopy, mass spectroscopy, mass spectroscopy in conjunction with gas chromatography, and nuclear magnetic resonance (NMR); sample sizes of a few mg are required for NMR.

VI SPECIFIC COMPOUNDS DETECTED AND IDENTIFIED USING THE CAM

A These compounds have been detected and identified.

α-conidendrin; o-nitro-chlorobenzene; phenyl ether; ABS detergents; nonionic detergents; 2-ethylhexyl phthalate; dieldrin; endrin; DDT; DDE; DDD; aldrin; heptachlor; heptachlor epoxide; BHC; parathion; methyl parathion; malathion; sevin; thiodan; chlordane; telodrin; technical and γ-chlordane; bis-(2-chloroethyl) ether; bis-(2-chloroisopropyl) ether; naphthalene; tetralin; styrene; acetophenone; ethyl benzene; 2-ethyl hexane; di isobutyl carbinol; phenyl methyl carbinol; 2-methyl-5-ethyl pyridine.

B These compounds have been detected but not identified specifically.

Nitriles; amines; phenols; acids; ketones; aldehydes; alcohols.

VII TREATMENT AND REMOVAL PRACTICES

A Plant treatment procedures, such as coagulation, sedimentation and filtration, are generally not too effective.

B Chemical Treatment

1 Copper sulfate used to control algae.

2 Oxidizing agents, such as chlorine, chlorine dioxide, ozone, and potassium permanganate, are used with varying degrees of success.

3 Activated carbon treatment removes organic substances by adsorption.

C Biological Treatment

1 Natural degradation in unsaturated soils and streams.

2 Biological oxidation of organic materials both in streams and acclimated systems.

VIII SUMMARY

The problems associated with trace organic contaminants in water are becoming more apparent as our needs and usage of water increase. The continued growth of the chemical industry, our increasing population, and the public's demand for more palatable water, emphasize even more the urgency of these problems. Developments have been made in the detection of these refractory materials and in their removal from water supply sources. It is apparent, however, that further advances in the collection, identification, and removal of these pollutants are needed to insure the public of high-quality water and water resources.

REFERENCES

1 Anon. Tentative Method for Carbon Chloroform Extract (CCE) in Water. JAWWA 54:223. 1962.

2 Ryckman, D.W., Burbank, NC., Jr., and Edgerley, E.Jr., New Techniques for the Evaluation of Organic Pollutants. Ibid. 56:975. 1964.

3 Ettinger, M.B. Developments in Detection of Trace Organic Contaminants. Ibid 57:453. 1965.

4 Robeck, G.G., Dostal, K.A., Cohen, J.M., and Kreissl, J.F. Effectiveness of Water Treatment Processes in Pesticide Removal Ibid. 57:181. 1965.

5 Anon. Taste and Odor Control - Chemicals and Methods. Taste & Odor Cont. J. 31(1):1. 1965.

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OPTIMIZING SAMPLING INTERVALS

I INTRODUCTION

A Large data systems are those that are hard to turn off.

B Optimizing sampling intervals in space fields is the same as optimizing in time series.

II PROCEDURES FOR OPTIMIZING SAMPLING INTERVALS

It may be possible to digitize the record at some interval larger than the recording interval. If so, future data collection and utilization procedures can be designed accordingly with consequent savings.

A Descriptive Models

1 Ranges, means, and quartiles. In pollution surveillance, the simplest and often most revealing statistic is the range. It is the extreme values that cause trouble.

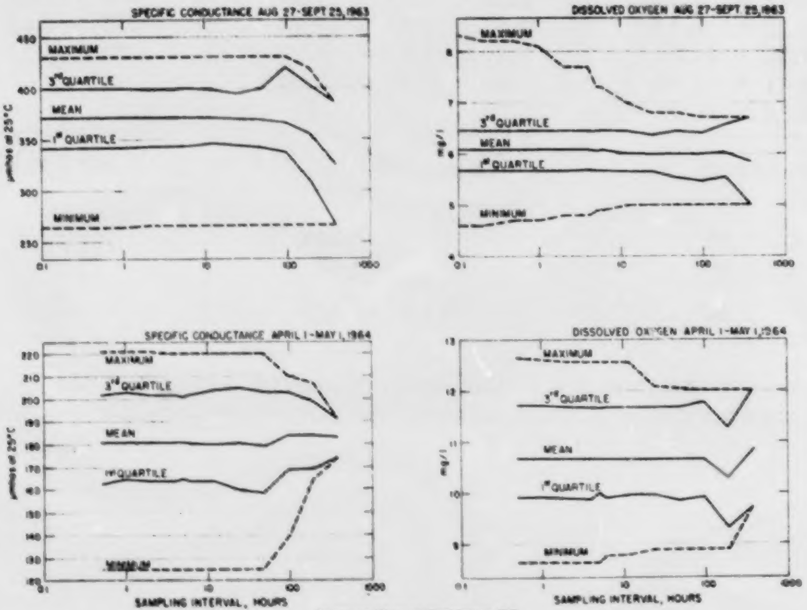
2 Frequency distributions. Plotting frequency distributions on normal probability paper is one of the easiest and most revealing exercises in evaluating environmental pollution data.

3 Comparisons of departures from continuous record. This is quite a bit of effort for what you get.

4 Spectral analysis. In recent years, the analysis of sequential data has been greatly facilitated by the use of power spectrum analysis. Spectral analysis information on the variance of a record.

It can be seen intuitively that any variance due to a secular trend in the record will be measured at lag 0. This is because a secular variation has an infinite period (zero frequency). This variance will, of course, be added to that due to harmonic motion.

Four steps in the computation of individual power spectra are as follows:



Potomac River at Washington, D.C.

Figure 1. Variation of mean, quartile, and extreme values of specific conductance and dissolved oxygen for selected sampling intervals during 31-day record.

C_0 ≡ Autocorrelation at lag 0	C_1 ≡ Autocorrelation at lag 1	C_2 ≡ Autocorrelation at lag 2
1.30 × 1.30 = 1.69	1.30 × 2.57 = 3.34	1.30 × 3.79 = 4.93
2.57 × 2.57 = 6.60	2.57 × 3.79 = 9.74	2.57 × 1.49 = 3.83
3.79 × 3.79 = 14.36	3.79 × 1.49 = 5.65	3.79 × 2.30 = 8.72
1.49 × 1.49 = 2.22	1.49 × 2.30 = 3.43	1.49 × 4.73 = 7.05
2.30 × 2.30 = 5.29	2.30 × 4.73 = 10.88	2.30 × . .
4.73 × 4.73 = 22.37	4.73 × . .	4.73 × . .
. . .	. . .	. . .
. . .	. . .	. . .
. . .	. . .	. . .
. . .	. . .	. . .
3.10 × 3.10 = 9.61	3.10 × 1.46 = 4.53	3.10 × 3.16 = 9.80
1.46 × 1.46 = 2.13	1.46 × 3.16 = 4.61	1.46 × 3.30 = 4.82
3.16 × 3.16 = 9.99	3.16 × 3.30 = 10.43	3.16 × 1.41 = 4.46
3.30 × 3.30 = 10.89	3.30 × 1.41 = 4.65	3.30 × 2.35 = 7.76
1.41 × 1.41 = 1.99	1.41 × 2.35 = 3.31	1.41
2.35 × 2.35 = 5.82	2.35	2.35
Sum = 1046.9	Sum = 804.67	Sum = 764.62
$\frac{1046.9}{145} = 7.22$	$\frac{804.67}{144} = 5.588$	$\frac{764.62}{143} = 5.347$
-5.90	-5.90	-5.90
$C_0 = 1.32$	$C_1 = -0.312$	$C_2 = -0.553$

[a] Number of observations = 145; mean of observed values = $\sqrt{5.90}$.

Table 1. Bacteriological Pollution at Selected Water Pollution Surveillance System Stations

Station	Mean coliform bacteria per 100 ml (Fig. 8)	Sources of pollution
Mississippi River at New Orleans, La.	1,500	Diffuse
Missouri River at St. Louis, Mo.	10,000	↓
Missouri River at Kansas City, Kan.	28,000	
Big Sioux River at Sioux Falls, S. Dak.	500,000	Dominant

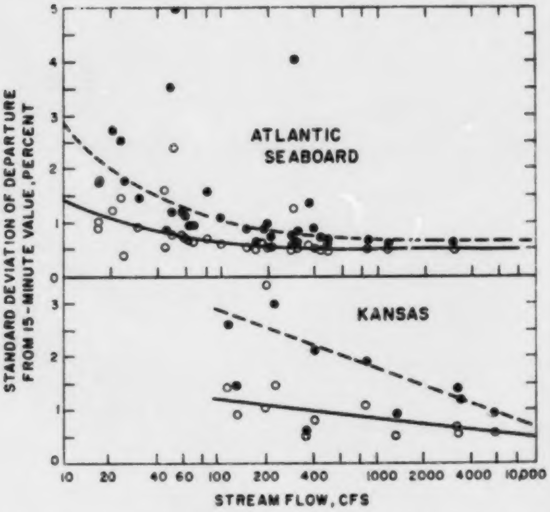


Figure 3. Comparisons of average daily flow computations from 30-minute (open circles) and 60-minute (black circles) data with computations from 15-minute data.

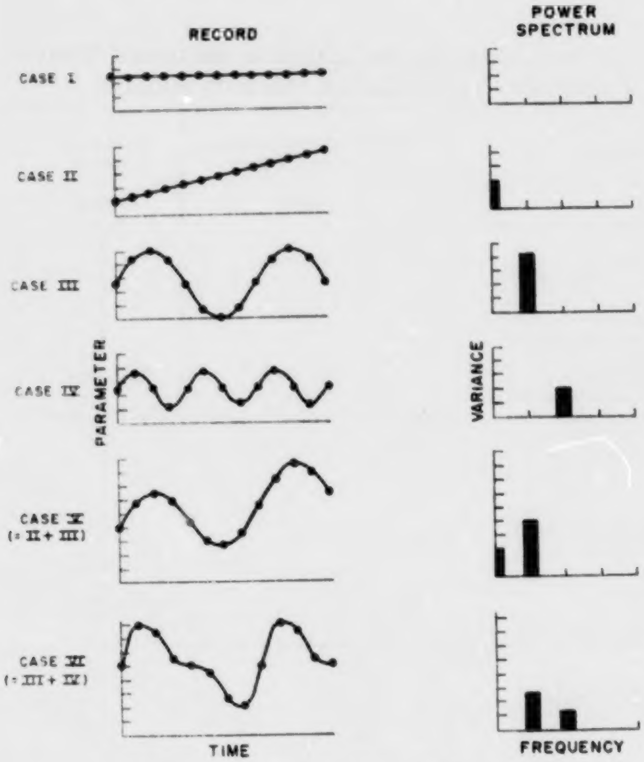


Figure 4. Spectral arithmetic

a The mean and the square of the mean of the record are determined.

b The autocorrelation function of the record is formed. This is the basic operation in spectral analysis, and it is described in detail below.

c The Fourier cosine transform for each autocorrelation is computed. This defines the difference between spectral analysis and standard Fourier analysis, because in the latter the Fourier transform is applied to the raw record rather than to the autocorrelation function. In spectral analysis, the Fourier transform serves to smooth out some of the fluctuations present in the autocorrelation function.

d A second weighting operation provides the estimate of spectral density.

The Autocorrelation Function. The autocorrelation function is obtained by first multiplying each number in the record. From the mean of the sum of these products is subtracted the square of the arithmetic mean of the entire series. In determining the autocorrelation at lag 0, the record is multiplied by itself so that the result is the variance as defined in statistics. The autocorrelations at lag 0, lag 1, lag 2, and lag 3 are computed as shown in Table 2.

The entire operation is expressed mathematically as

$$C_r = \frac{1}{n-r}\sum_{1}^{n-r} x_i x_{i+r} - \left[\frac{1}{n}\sum_{1}^{n} x_i\right]^2 \qquad (1)$$

Table 2. Typical computation of Autocorrelation Function[a]

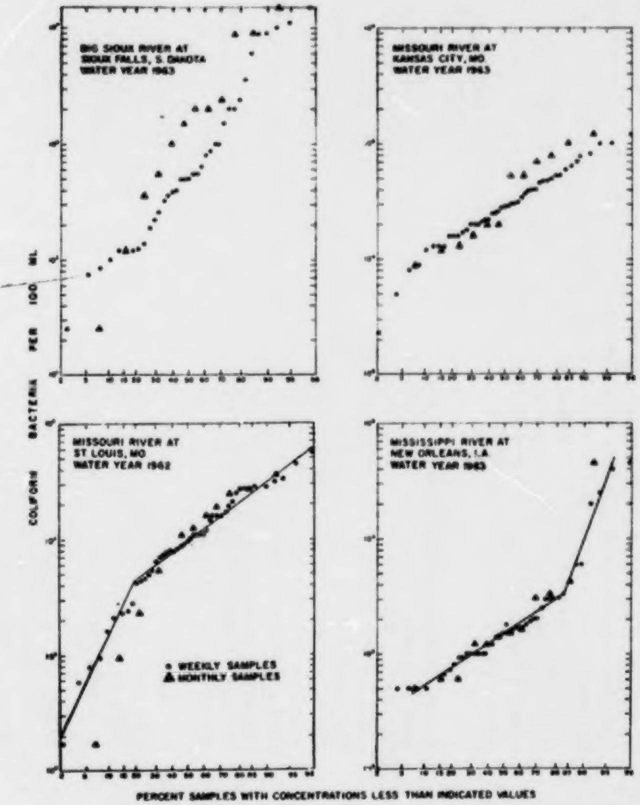


Figure 2. Frequency distribution of coliform bacteria in surface water

in which

C_r = autocorrelation at lag r
x_t = record value at t
t = 0, 1, 2 . . . n = sequential index of values
r = 0, 1, 2 . . . m = lag number
m = total number of lags.

In the simple case of a pure sine wave, the computation is physically analogous to looking at a white picket fence through one of a series of vertical gratings. Both the fence and the gratings are constructed so that the bar width always equals the slot width. The pickets are a square-wave approximation of the sine wave. The resolution of detail with which the fence may be seen is a function of the spacing between grates. Similarly, the effective resolution (R) of the sine wave is a function of the number of lags (m) used for computing the autocorrelation function and of the sampling interval (ΔT), where $R = 1/2m\Delta T$.

If the spacing of the grates is too large, some of the pickets will not be seen. The total amount of light seen by the viewer will be less than the amount reflected by the fence. When the grating with the optimum spacing is selected, the fence can be described precisely. This also happens when the optimum number of lags is applied to the sine wave record.

As the grate spacing continues to be decreased, all of the pickets can still be seen, but some of the light is again lost to the viewer. Soon, only half the reflected light is available, and eventually the visual resolution of the fence is destroyed entirely because the grating dimensions approach those of light waves. Here, interference and diffraction patterns are set up. Something comparable, although not strictly analogous, happens when the autocorrelation is computed to too many lags for the particular record length. The computation becomes unstable.

A common rule of thumb is that the number of lags should not exceed 10% of the number of data points. This appears to be unduly conservative, Since analyses of estuarial data have successfully used lagged computations numbering up to 30% of the number of data points.

Fourier Transform and Smoothed Spectrum

The Fourier cosine transform is calculated as

$$V_r = \frac{k}{m}\left[C_0 + C_m \cos r\pi + 2\sum_{q=1}^{m-1} C_q \cos\frac{qr\pi}{m}\right] \qquad (2)$$

in which

V_r = Fourier cosine transform of the autocorrelation at lag r

q = lag number having values between 1 and m-1 and
k = a constant

$$\left.\begin{array}{lll} k = 1 & \text{for} & r = 1, 2 \cdots m - 1 \\ k = \frac{1}{2} & \text{for} & r = 0 \\ & & r = m, \end{array}\right| \qquad (3)$$

and the other letters have the definitions previously given.

The smoothed estimate of spectral density is obtained from a final weighting function, expressed mathematically as

$$U_0 = 0.54V_0 + 0.46V_1 \qquad (4)$$

$$U_r = 0.23V_{r-1} + 0.54V_r + 0.23V_{r+1} \qquad (5)$$

for r = 1, 2, 3, . . . m - 1, and

$$U_m = 0.46V_{m-1} + 0.54V_m \qquad (6)$$

in which U_0, U_r, U_m equal the power spectrum estimates corresponding to the respective lags, and the remaining symbols have the meanings previously assigned.

Figures 5 and 6 show that, even in tidal estuaries where there are continuous changes in velocities, a 2-hour sampling interval is adequate for a program of monthly data analysis.

5 **Aliasing.** The designs of both the sampling interval and the subsequent statistical or spectral analysis require consideration of aliasing. Aliasing is defined graphically in Figure 7 and results from the high-frequency events that add variance to the record but that

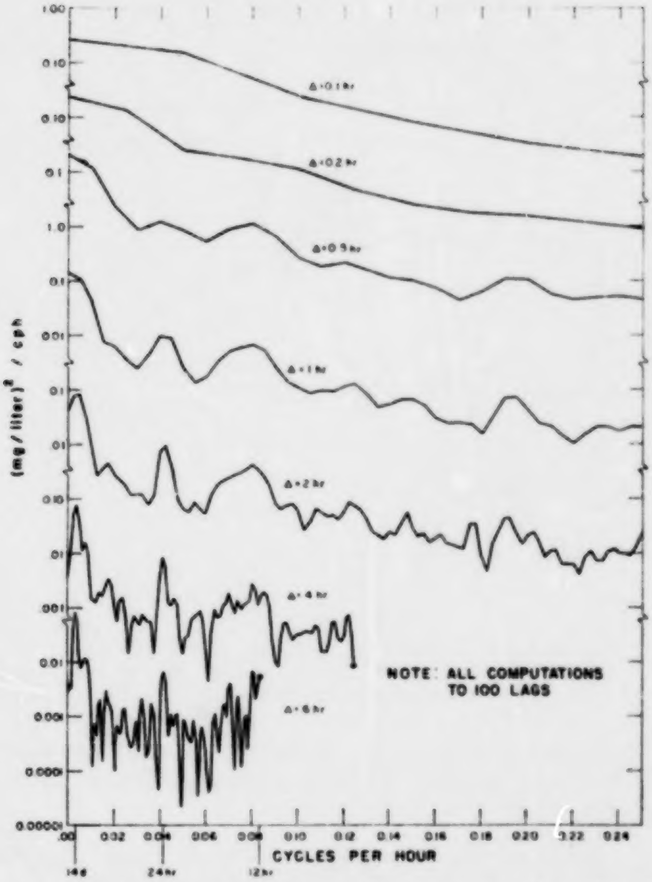


Figure 5. Estimates of spectral density: dissolved oxygen, Potomac River at Washington, D.C., August 27-September 26, 1963 (Δ indicates sampling interval)

are not "seen" by the particular sampling interval. Figure 7 illustrates how the variance from this type of event is folded into the record and reappears at a lower frequency. Where the period of the high-frequency event is known, the period of the aliased record can readily be determined analytically.

Inspection of Figure 7 reveals that any cyclic event that occurs at a period less than twice the sampling interval will result in aliasing. Where the period equals twice the sampling interval, the event will never be seen. Only when the period exceeds twice the sampling interval can the event be measured. As the period of the cyclic event approaches a value of twice the sampling interval, the record length necessary to describe the event increases. The corresponding frequency, fn, which limits the events

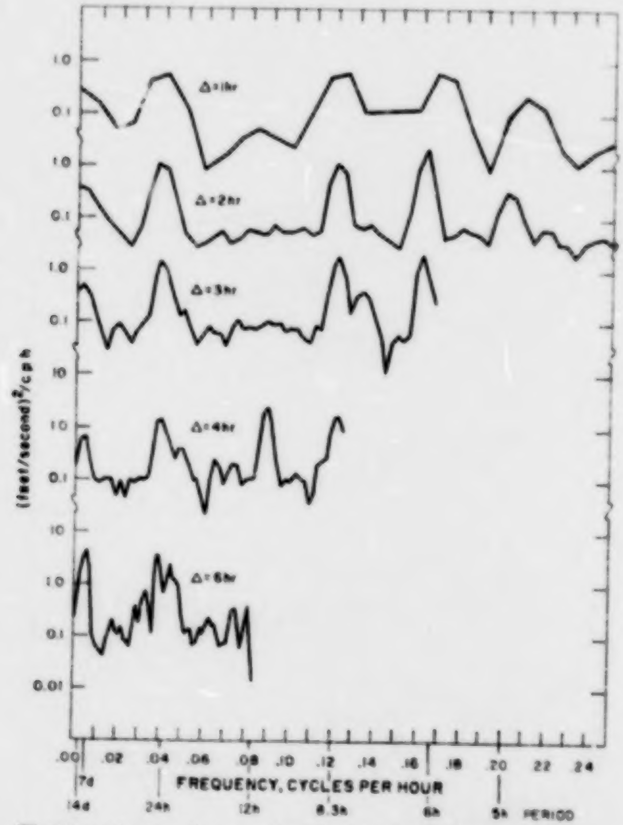


Figure 6. Estimates of spectral density for surface currents, Sacramento River at mouth, August 1-September 1, 1963 (Δ indicates sampling interval)

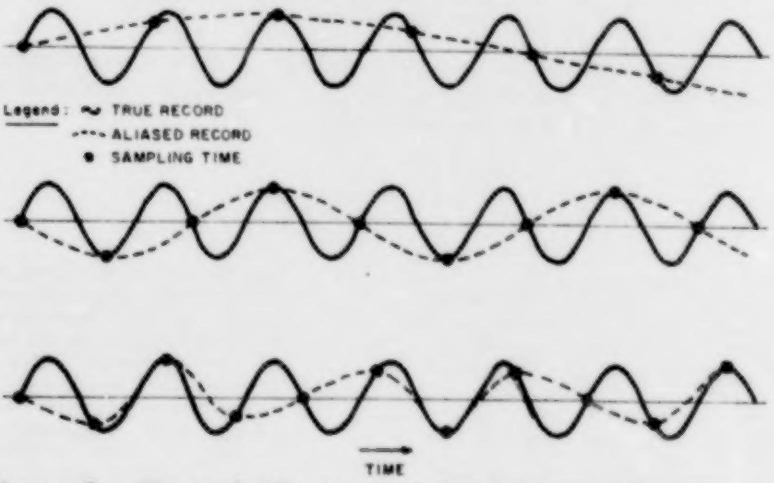

Legend:
TRUE RECORD
ALIASED RECORD
SAMPLING TIME
TIME

Figure 7. Typical effects of observing periodic events at intervals of more than one-half the period

seen by a particular sampling frequency, fs, is known as the Nyquist frequency, fn = 1/2 fs.

B Predictive or Control Models

1 Automatic monitoring does not save money.

2 In pollution surveillance where an automatic monitor is the sole basis for identifying random slugs of discharges of waste, continuous monitoring is meaningful only at the discharge point.

3 A corollary of the aliasing problem is that data collected at less than half the interval required for changing plant process cannot be "seen" by the plant.

4 Continuously collected environmental data are being used to control stack discharges from the Enrico Fermi Atomic Power Plant near Detroit. Even here, with a plant which is inherently responsive to changes in demand, a 24-minute control interval is used.

5 The ultimate in optimizing sampling intervals for pollution control is probably that used in the Ruhr Valley where they sample twice yearly.

REFERENCES

1 Carter, R. W., Anderson, W. L., Isherwood, W. L., Rolfe, K. W., Showen, C. R., and Smith, W. Automation of Stream Flow Records. Circular 474, Geol. Survey, U. S. Department of the Interior, Washington, DC (1958).

2 Gill, G. C., and Bierly, E. W. A Meteorologically Operated Stack Control System. Journal of Applied Meteorology, Vol. 2. No. 4. 431-439. (1963).

3 Gunnerson, C. G. Optimizing Sampling Intervals in Tidal Estuaries. Journal Sanitary Engineering Division, Proc. American Society of Civil Engineers. Vol. 92, No. SA2. 103-125. (1966)

4 Gunnerson, C. G. Hydrologic Data Collection in Tidal Estuaries. Water Pollution Research. Vol. 3, No. 2. 491-504. (1967)

5 Gunnerson, C. G. Optimizing Sampling Intervals. Proceedings, IBM Scientific Computing Symposium on Water and Air Resource Management, October 23-25, 1967. IBM Corporation, White Plains, NY. 115-140. (1968)

6 Kneese, A. V. The Economics of Regional Water Quality Management. The John Hopkins Press, Baltimore, MD. (1964)

7 Wastler, T. A. Application of Spectral Analysis to Stream and Estuary Field Surveys - I. Individual Power Spectra. Publication No. 999-WP-7, U. S. Public Health Service, Washington, DC. (1963). Note: This is out of print. A revised and expanded version is scheduled for early publication by the Federal Water Pollution Control Administration.

This outline was prepared by Charles G. Gunnerson, Deputy Chief, Research and Development, Solid Wastes Program, Environmental Control Administration, Consumer Protection and Environmental Health Service, PHS, DHEW.

BIBLIOGRAPHY ON SPECTRAL ANALYSIS AND RELATED STUDIES

1 Barber, N. F. Experimental Correlograms and Fourier Transforms. Pergamon Press, Inc. New York, NY. 1961.

This is an excellent introduction, useful to the engineer or physical scientist, which clearly shows the physical significance of Fourier transforms, correlograms, and power spectra. The underlying theory and a wide variety of mechanical, optical, and electrical analogs are reviewed.

2 Barber, N. F., and Tucker, M. J. Wind Waves. The Sea. Vol. I, edited by M. N. Hill, Interscience Publishers, Inc. New York, NY. 664-699. 1962.

Presents example of need for and results from spectral analysis.

3 Blackman, R. B., and Tukey, J. W. The Measurement of Power Spectra. Dover Publications, Inc. New York, NY. 1958.

This is the standard reference for the theory and application of spectral analysis.

4 Cartwright, D. E. Analysis and Statistics. The Sea. Vol. I, edited by M. N. Hill. Interscience Publishers, Inc. New York, NY. 567-589. 1962.

Summarizes statistical formulation of sea waves and introduces concepts of spectral analysis.

5 BMD-Biomedical Computer Programs, edited by W. J. Dixon, Health Sciences Computing Facility. Dept. of Preventive Medicine and Public Health School of Medicine. Univ. of California. Los Angeles, California. January 1, 1964.

Includes digital computer programs for individual power spectra, cross spectra, and other time-series analyses.

6 Dronkers, J. J. Tidal Computations. Interscience Publishers, Inc. New York, NY. 1964.

A basis and complete treatment of tidal hydrodynamics, harmonic analysis, and computational methods.

7 Holloway, J. L., Jr. Smoothing and Filtering of Time Series and Space Fields. Advances in Geophysics. Vol. 4. Academic Press. New York, NY. 351-389. 1958.

Reviews and presents basis for selection from a variety of methods for smoothing and filtering serial data.

8 Kinsman, B. Wind Waves, Their Generation and Propagation on the Ocean Surface. Prentiss-Hall. Englewood Cliffs, NJ. 1964.

An outstanding description of the theory and application of spectral analysis.

9 Munk, W. H., Snodgrass, F.E., and Tucker, M. J. Spectra of Low-Frequency Ocean Waves. Bulletin of the Scripps Institute of Oceanography. Univ. of California. Los Angeles, California. Vol. 7. No. 4. 283-362. 1959.

10 Panofsky, H. A., and Brier, G. W. Some Applications of Statistics to Meteorology. Pennsylvania State Univ. Press. University Park, PA. 1958.

Chapter VI is an excellent introduction to the subject.

11 Time Series Analysis, edited by M. Rosenblatt. Proceedings of the Symposium at Brown University. Providence, RI. June 11-14, 1962. John Wiley & Sons, Inc. New York, NY. 1963.

Includes papers on a broad range of topics, including statistical theory, spectral analysis, and applications to geophysical, engineering, economic and biological problems.

12 Snodgrass, F. E., Munk, W. H., and Miller, G. R. Long-Period Waves over California's Continental Borderland-I: Background Spectra. Journal of Marine Research. Vol. 20. 3-30. 1962.

Presents an example of results derived uniquely from spectral analysis of ocean waves.

13 Southworth, R. W. Autocorrelation and Spectral Analysis. Mathematical Methods for Digital Computers, edited by A. Ralston and H. S. Wilf. John Wiley & Sons, Inc. New York, NY. 213-220. 1960.

Presents digital computer program for determining autocorrelation function.

14 Stommel, H. Varieties of Oceanographic Experience. Science. Vol. 139. 572-576. 1963.

Presents magnitudes and interrelationships of time and space characteristics for both seal level and ocean currents.

II CHROMATOGRAPHIC METHODOLOGY

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CARBON ADSORPTION METHOD APPLIED TO ISOLATION OF PESTICIDES FROM WATER

I INTRODUCTION

The purpose of this discussion is to present the concept of the carbon adsorption method (CAM) as it is applied to the isolation of pesticides, particularly the chlorinated hydrocarbon type, from water. Some of the principles, limitations, and utility of the method are presented. The method as described below is considered a qualitative technique. It was not designed specifically for the isolation of pesticides. Rather, it was designed as a general technique for isolation of a broad spectrum of organic chemicals from water. Coincidentally, we are able to isolate many important pesticides.

The CAM has been used as a routine surveillance tool by the Federal Water Pollution Control Administration's Water Quality Activities since 1957. Its value in this work has been proven. However, there are many factors to be considered before deciding to adopt the CAM. These factors will be discussed below.

The elaborate equipment described below may not be needed for specific problems. Small scale equipment may be applicable for specific problems. Bench tests should be carried out on small columns to evaluate the applicability of the method to the problem.

Details of the equipment used by FWPCA for collection and isolation of organics from water are not given here. This information is available in USDI Publication WP-22 (1966).

II COLLECTION OF SAMPLE

A Carbon Adsorption Cartridge

B Sampling Equipment

1 High flow

2 Low flow

3 Finished water

C Total Volume and Rate of Flow

1 Total volume

5000 gallons
1000 gallons
300 gallons

2 Rate of flow

0.5 gpm
0.25 gpm
0.03 gpm

III PREPARATION OF THE SAMPLE

A Drying

B Extraction

C Separation and Clean-up

1 Column chromatography

2 Thin-layer chromatography

IV DETERMINATION

A Gas Chromatography

1 Electron capture

2 Microcoulometric titration

V SENSITIVITY LIMITS

All data derived by use of the CAM must be considered qualitative. Numerical values attached to results thus obtained are considered to be minimum values.

A Practical Limits

1 Gas chromatographic determination

Electron capture gas chromatography is capable of detecting absolute quantities of less than 1 nanogram (10^{-9}gm). The microcoulometric titration system (Model C-200, Dohrmann Instruments Company) provides a sensitivity of 5 to 10 nanograms for many pesticides.

Consider a 5000-gallon (20,000 liters) sample. The dilution factors involved in the analysis are 10 and 100. Thus:

1 ng × 10 × 100 = 1000 ng or 1 µg

The limit then is 1 µg/20,000 liters or 0.00005 µg/liter for the electron capture determination. The limit for the microcoulometric determination is estimated at 0.00025 to 0.0005 µg/l. In practical application the lower limit is conservatively estimated at 0.001 µg/l or 1 ppt for both systems.

B Theoretical Limits

Assuming freedom from interferences and with considerable additional effort, it is theoretically possible to detect concentrations of 0.00000005 to 0.00000025 µg/l in a 5000-gallon sample.

VI CONTROL OF INTERFERENCES

Control and awareness of interferences is extremely important in trace analysis. The great sensitivity of gas chromatographic instruments in pesticide analysis emphasizes this importance. It is essential, therefore, that the analyst take the ultimate precautions to minimize the problem.

There are several measures, preventive and remedial, that need to be taken in controlling interferences.

A Preventive

Examples of this type of measure are:

1 Storing carbon in sealed containers.

2 Prefiltering air which passes through carbon drying ovens.

3 Prohibiting the use of sprays for pest control in areas where the analyses are carried out.

B Remedial

1 Proper cleaning of all collection equipment prior to use.

2 Carbon blank determinations.

3 Solvent blank determinations and redistillation when required.

4 Silica gel blank determinations - column and TLC.

5 Proper cleaning and when possible, muffling of glassware.

All control determinations must be made under the conditions of the analysis, including the final step of determination on the gas chromatograph.

VII LIMITATIONS OF THE METHOD

There are certain limitations such as the cost of installation and maintenance of the elaborate collection equipment to be considered. The time required to collect a sample sometimes limits its usefulness. More important, however, are the limitations for quantitative recovery. Some of the factors which limit the method to a qualitative technique are listed below:

A Adsorption Efficiency

The adsorption efficiency may be affected by: the type of carbon, the rate of flow-contact time, the total volume of water sampled, preferential adsorption of some compounds, changes in selective adsorption during the collection of sample, overloading the carbon, pH, temperature, and mineral constituents in the water.

B Desorption Efficiency

The desorption efficiency may be affected by: the type of carbon, extracting solvent, extraction time, irreversible adsorption of some compounds.

C Chemical and Biological Decomposition During Adsorption and/or Desorption

D Prior Removal by Sand Pre-filter

The use of the new low flow, low throughput sampling units serves to minimize some of the limitations.

VIII ADVANTAGES OF THE METHOD

In spite of the foregoing limitations, the CAM has proved very useful in the continuous surveillance of organic contaminants in water. It provides two important advantages:

A A Continuous Integrated Sample Over a Period of Time

B A Large Volume Sample (Up to 5000 gal) Which Provides a Larger Quantity of Sample for Analyses

IX SUMMARY

The CAM as applied to pesticide analysis has been described. The need for control of interferences and the limitations of the method have been recognized. These variables suggest caution in the interpretation of data obtained by this technique. However, the method has important advantages over the small grab sample method which make it useful as a routine surveillance tool as well as in certain specific pollution problems.

REFERENCES

1 Braus, H., Middleton, F. M. and Walton, G. Anal. Chem., 23, 1160. 1951.

2 Middleton, F. M. and Lichtenberg, J. J. Ind. and Eng. Chem., 52, 99A. 1960.

3 Rosen, A. A. and Middleton, F. M. Anal. Chem., 31, 1729. 1959.

4 Breidenbach, A. W. and Lichtenberg, J. J. Science, 141, 899. 1963.

5 Breidenbach, A. W., Lichtenberg, J. J., et al. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. USDI Publication WP-22, Washington, D. C. 1966.

6 Weaver, L., et al. Public Health Reports, 80, 481. 1965.

7 Breidenbach, A. W., et al. Chlorinated Hydrocarbon Pesticides in Major River Basins 1957-1965, to be published in Public Health Reports.

8 Green, R. S., Gunnerson, C. G., and Lichtenberg, J. J. Pesticides in Our National Waters. Presented at American Assoc. for the Advancement of Science Symposium on Agriculture and the Quality of Our Environment. Washington, D. C. Dec. 27, 1966.

9 Hassler, J. W. Activated Carbon. Chemical Publishing Company, N. Y. 1963.

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METHODS OF RECOVERING ORGANIC MATERIALS FROM SURFACE WATERS

Methods of recovering organic materials from water may be classified according to the degree of concentration required before the desired analytical procedure can be applied.

I CONCENTRATED SOLUTIONS

When the concentration is high and/or the methods are sufficiently sensitive (i.e., when only a minor degree of concentration is involved), the following methods should be considered.

A Liquid-liquid extraction usually involves water and an immiscible organic solvent. Solvents should be investigated in the series of increasing polarity:

Aliphatic hydrocarbons
Aromatic hydrocarbons
Ethers
Chlorinated compounds
  (CCl_4, $CHCl_3$, CH_2Cl_2)
Esters
Alcohols, amines, acids, etc.

Because of hydrolysis or decomposition, pH may be of critical importance; this point will be discussed at length in the discussion of Analytical Procedures. Inorganic salt concentration may also be important.

Remember the concept of the partition coefficient:

$$K = \frac{C_s}{C_w}$$

where K is the partition coefficient,

Cs is concentration in the extracting solvent

Cw is the concentration in water.

Continuous extractors must be used where the K value is not favorable to the extracting solvent. Continuous batch and counter-current extractors may be used.

Separatory funnels are available in a variety of sizes. Batch continuous extractors are commercially available in sizes up to one liter or so, and can be readily assembled in larger sizes. Continuous counter-current extractors are convenient for up to 10-20 gallons.

B Steam distillation can be used to strip a sample of small amounts of volatile organic compounds; in this case, the distillate usually must be further concentrated by liquid-liquid extraction. The organic compound should have at least a moderate vapor pressure at 100°C and should be almost insoluble in water.

A variation of this method is simple evaporation to concentrate non-volatile organic compounds.

C Precipitants may be used: silver salts of acids, chloroplatinates or tetraphenyl boron, derivatives of amines, phenylhydrazine derivatives of ketones and aldehydes, etc.

D Ion exchange may be used to concentrate acids and bases. The only limit to the volume of water to be filtered is the amount of inorganic salts in the water; these inorganic salts usually use up the exchange capacity too rapidly to make this method practicable.

II VERY DILUTE SOLUTIONS

To concentrate extremely dilute solutions, the carbon filter is the most useful method. The organic matter is adsorbed from aqueous solution and desorbed by an organic solvent. The great advantage is the large amount of water that can be put through a small filter; the disadvantages lie in the lack of quantitative adsorption and desorption.

A The Adsorption Process

1 The adsorption process involves an equilibrium in solution.

$$\text{Adsorbed} \rightleftharpoons \text{Unadsorbed}$$

2 The Freundlich Isotherm is often useful in evaluating adsorption behavior.

$$\frac{x}{m} = kc^{\frac{1}{n}}$$

Units may be chosen for convenience, the larger the $\frac{1}{n}$, the stronger the adsorption. The constant k gives the $\frac{x}{m}$ value at unit concentration.

x = weight of adsorbed compound
m = weight of carbon
k = constant = x/m when c is unity
c = equilibrium concentration of organic compound in the liquid phase; it is measured directly.
$\frac{1}{n}$ = constant determined by the slope of the isotherm

3 General Rule: Non-polar compounds are strongly adsorbed and polar compounds weakly adsorbed from water (e.g., mineral oil is more strongly adsorbed than glycine).

4 Adsorption will depend on the type of carbon, grain size, pH of solution, polarity of adsorbate, nature of the solvent, temperature, etc. If powdered carbon is not used, the column length and contact time must be chosen so as to permit efficient adsorption.

5 Desorption is simply the reverse reaction in the adsorption equilibrium and is influenced by the same factors as adsorption. It is usually necessary to use an organic solvent for desorption with an extended period of extraction.

B Setting up the Carbon Filter

1 In the next section is a description of the procedure used in setting up and running carbon filters in our laboratory. However, it should be understood that elaborate apparatus is not required and that excellent results can be obtained with a piece of glass tubing closed at either end by a rubber stopper in which is inserted a piece of smaller glass tubing. Such apparatus is adequate for testing small volumes (e.g., sewage or concentrated industrial wastes), although not convenient for handling thousands of gallons of water.

2 Wherever the investigation is concerned only with certain specific compounds, bench tests in small columns should be carried out in order to evaluate the performance to be expected from the filter.

III INSTALLATION OF EQUIPMENT AND COLLECTION OF CARBON FILTER SAMPLES

A The carbon filter consists of a piece of pyrex glass pipe, 3" in diameter and 18" in length. The ends are fitted with brass plates and 3/4" galvanized nipples. A stainless steel screen is fixed in a neoprene gasket at both ends.

B Presettling, Prefilter, and Backwash

River water will frequently clog the carbon filter before the desired amount of water has been sampled. It is necessary to remove sufficient turbidity to permit the required amount of water to pass through the unit. This may require a presettling tank, and a prefilter containing sand and gravel. For waters having less than 100 ppm of turbidity, a presettling tank generally is not required. Tap water, of course, requires no prefiltering and may be passed directly into the carbon filter.

C Presettling Tank

A standard hot water tank connected with the inlet at the bottom and outlet at the top, with a clean-out tap at the bottom, can serve as a presettling tank. The outlet connects to the prefilter containing sand and gravel. The hot water tank should be flushed at frequent intervals to prevent large accumulations of solids. Open settling tanks can be used if care is taken to prevent long detention times and biological action. With open tanks a pump will be required to move the water through the filter.

D Sand Prefilter

The sand prefilter consists of a standard piece of steel pipe, 3" in diameter and 3 feet long, threaded at both ends and equipped with 3"×1" reducer couplings. Two disks of stainless steel screen are fitted to the inside diameter of the pipe. A simple way to prepare and hold the screen in place is to cut out a disk about the size of the outside diameter of the reducer and then push it into place so that is is below the threads. The screen is held tightly against the inside of the reducer. The pipe is packed with 6" of 1/8" gravel, 24 inches of 0.6 - 0.8 mm sand, and another 6" of 1/8" - 1/4" gravel. No free space is left in the pipe. The gravel should be packed by jarring while fitting the pipe. This arrangement provides a strainer rather than a filter with a movable bed. With such an arrangement, backflushing can be done without disturbing the filter. The construction details are shown in Figure 1. The dimensions may be varied according to local conditions.

IV FILTER ARRANGEMENT

A The presettling tank, the sand prefilter, and the carbon filter should be installed at the most convenient source of raw water. If less than 15 psi pressure is available, it may be necessary to pump the water through the system. A schematic drawing of a workable system is shown in Figure 2. Exact lengths of pipes, etc., are not given since these will vary with the local situation. Both the sand and carbon filters should be connected with unions at both ends for easy removal. A typical installation is shown in Figure 2.

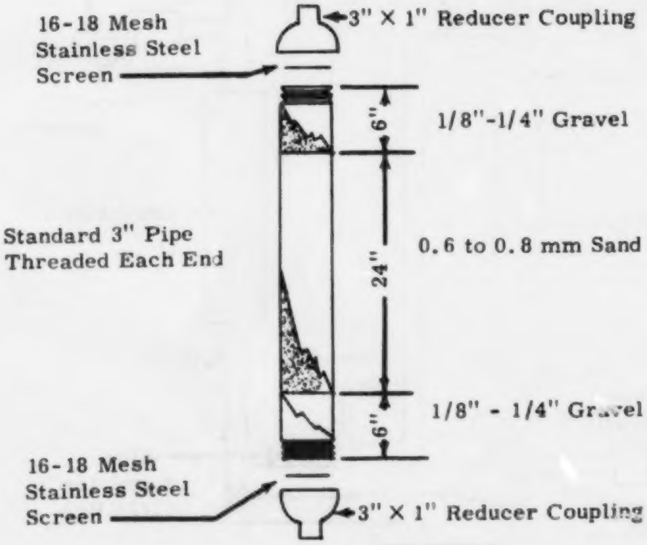

FIGURE 1 - Details of Sand Prefilter

B The raw water passes into the bottom of the sand prefilter, around into the bottom of the carbon filter. When the rate of flow through the system falls below 1/4 gpm, backwashing of the sand filter is necessary, using a high pressure source of water. A clean hose is connected to the top of the sand filter, the valve to the carbon filter is closed and the drain valve on the sand filter is opened. The sand is back-flushed until the water coming out is clear. The length of time between backwashings will vary. On the Missouri River, for example, it has been found that once every 24 hours is sufficient. After backwashing, connect the system as before and continue the sampling. Be sure to disconnect hose at top of sand filter after backwashing. A pressure gauge is inserted in the system to indicate when clogging is taking place in the carbon filter. Total pressure in the filter should not exceed 50 psi.

C A water meter located at the end of the system to prevent excessive fouling of the meter, is used to measure the volume of water samples. It is good practice to disassemble and clean the meter thoroughly after each run. A disk-type meter, 5/8"×3/4", is fairly satisfactory. A valve following the meter serves to throttle the flow, if necessary. A flow-regulating device may also be used for this purpose. The complete installation is shown in the schematic diagram in Figure 2.

D Fine carbon washes out of the filter when it is first started. A few gallons of water can be passed through the top connection and through the carbon filter drain before cutting in the meter to keep the meter free of carbon. The hose connection and drain on the carbon filter can be used to back-flush the carbon should it become clogged. This is a last resort and should only be used if absolutely necessary.

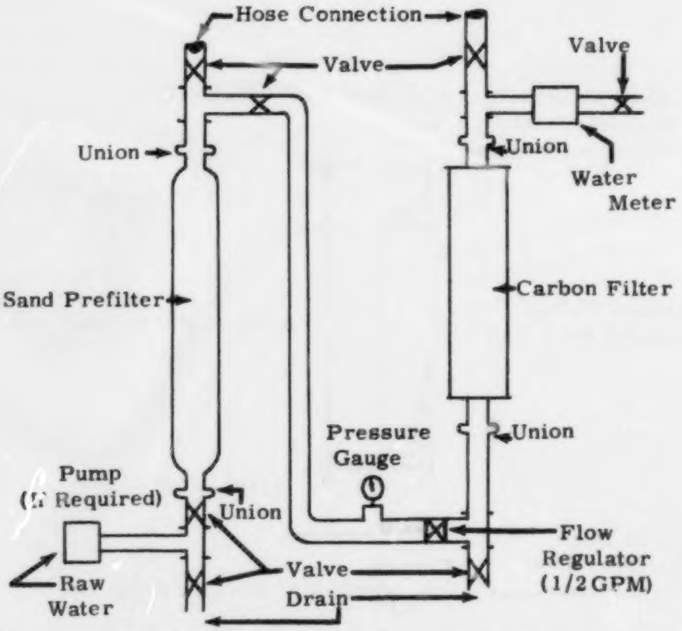

Figure 2 - Schematic Diagram of Piping Installation for Sand Prefilter and Carbon Filter

E The system outlined is not intended to remove all traces of turbidity from the water before passing through the carbon filter. Its purpose is to take out gross materials and most organisms, and to permit the required volume of water to pass through the carbon. The valve nearest the bottom of the sand filter is to be closed when it is desired to obtain a sample of raw water directly for other analyses. The drain valve is opened and, after flushing, the necessary raw water sample is collected.

F Pumping

1 If adequate pressure is not available for sampling raw water, it is necessary to pump through the filter system. It is important that the pump used should not contaminate the sample through oily packing or other sources. New pumps are oftentimes grease-coated and should be thoroughly cleaned before being put into service. If a lift of more than 12 feet is required, it will be necessary to use a jet-type pump. A model suitable for the conditions should be selected.

2 Before a new pump is put into service, it should be thoroughly flushed with hot water containing a little detergent. The pump should be operated against the minimum amount of head required to get the water through the filter. This may require by-passing of part of the flow.

G Collection of Sample

1 With fairly concentrated samples (sewage, industrial wastes, etc.), a small filter and a few gallons of water may be sufficient. Generally, for river sampling, water should be passed through a large filter at a rate of 1/4 - 1/2 gallon per minute, until up to 5000 gallons or more have been filtered. With highly turbid waters clogging may occur earlier. Although a suitable sample can be obtained sometimes with several hundred gallons of river water, it is desirable to filter a minimum of 2000 gallons if at all possible. A flow-regulating device set at 1/4 or 1/2 gpm can be placed in the system ahead of the carbon filter. A suitable unit can be obtained from Dole Valve Company, 6201 Oakton Street, Morton Grove, Illinois. These devices generally need a minimum pressure of 15 psi for proper operation.

2 Since the purpose is to get a quantitative measure of organics in water, it is very important to have accurate flow measurements. If difficulties in flow occur, this should be noted on a log sheet. Meter readings should be recorded daily on log sheets and should be designated either in GALLONS or CUBIC FEET. If a satisfactory meter can not be obtained, the flow rate for a set volume (1 or 2 liters) can be determined at regular intervals.

H Precautions

The purpose of the carbon filter is to adsorb small amounts of impurities from the water. It is important to avoid contamination of the carbon from other sources. Hence, the following should be observed.

1 New galvanized fittings are usually coated with oil or grease. The oil should be removed with a wash in kerosene followed by a detergent wash before such fittings are used for making connection to the filter.

2 Ordinary organic pipe jointing compounds should not be used. Red lead (lead oxide) mixed to a paste with water, can be used for this purpose.

3 Plastic hose is to be avoided. If rubber hose is used in any connections it should be flushed thoroughly before being connected to the filter. Copper tubing is ideal for connections.

I After the required volume of water has been run through the filter, the carbon is removed, dried, and extracted. The wet carbon is spread out in a thin layer on a metal sheet and air-dried for several days. The time needed for drying increases with the thickness of the layer. Warm air can be passed over the surface of the carbon to hasten the drying, but with the risk of driving off weakly held volatile substances.

V LABORATORY TREATMENT OF SAMPLES OBTAINED BY THE CARBON ADSORPTION TECHNIQUE: EXTRACTION PROCEDURES

The sample is collected by passing approximately 5000 gallons of water through a carbon adsorption unit. The unit is shipped to SEC, along with a daily record of the sampling activity and the carbon treated as described below.

A Preliminary Treatment

1 Records

- a Log sample in. List date received. Assign it a number.
- b Send daily record sheet to the laboratory where pertinent data is recorded on a data card (i.e., source, location, dates sampled, date received and total flow).

2 Drying

- a Remove wet carbon from the tube and dry it on copper, brass or stainless steel trays in an oven at 40°C. (n.b. Air circulated through drying cabinet should be prefiltered through carbon to prevent adsorption of foreign materials.) Drying requires about two days.
- b Store carbon samples in one gallon paint cans tightly closed.

B Solvent Extraction of Carbon

It is necessary that blanks be run on all solvents and on the carbon used for the collection of the sample.

1 Packing the extractors

- a Filter paper at bottom of soxhlet.
- b Glass wool (pre-extracted) 3" in depth to prevent carbon fines from passing into the pot. Add chloroform to wet the wool.
- c Carbon is added and packed. (n.b. Do not pack too tightly.)

2 Chloroform extraction

- a Add chloroform - about two cylinder volumes.
- b Extract for 35 hours.
- c Siphon and blow the bulk of the chloroform into the pot with air.
- d Remove the flask containing chloroform solution from the system. Concentrate to 250 ml by distillation and filter into a 300 ml Erlenmeyer flask. Evaporate to about 20 ml on a steam bath with a stream of air and transfer to a tared 5 dram vial. The remaining solvent is evaporated at room temperature without an air jet and the weight obtained.

3 Alcohol extraction

- a Remove residual chloroform from the carbon by one of the following methods.
    - 1) Blow warmed (40° C) prefiltered air upward through the carbon for about four hours.

2) Leach residual chloroform off the carbon with ethyl alcohol (95%) and distill off the chloroform alcohol mixture. Repeat until virtually pure alcohol remains.

3) Remove the carbon from the soxhlet and air dry on trays. When dry, repack into soxhlet as before. (Chloroform vapors must be disposed of.)

b Add sufficient ethanol (about two cylinder volumes) and extract for 24 hours.

c Concentrate the alcohol solution as in the case of chloroform, except that the final drying can be carried out on a steam bath with a stream of air.

The first method listed is the most satisfactory for our work here, mainly because a minimum of supervision is required and chloroform vapors are exhausted out the hoods.

C Extractable Materials

1 Calculation of concentrations

On most waters, it is convenient to compute the recovery in parts per billion (ppb) (i.e., $\mu g/l$), using the following formula.

$$\text{ppb} = \frac{\text{grams recovered} \times 10^6}{\text{gallons filtered} \times 3.785}$$

2 Infrared spectra

Infrared spectra are routinely run on both the chloroform and alcohol extracts.

D Special Applications

Other solvents may find use in performing extractions. If special applications are needed, a testing program is necessary to establish what solvents may best be used.

E Adsorption and Desorption

The effectiveness of adsorption and desorption varies for different materials and should be considered in interpretation of the results.

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PRELIMINARY SEPARATION OF EXTRACTS

I INTRODUCTION

As a general principle, it may be stated that the more sensitive modern analytical techniques (gas and other forms of chromatography, spectrophotometry, etc.,) require fairly pure samples to begin with. Also as a general principle, it may be said that the more widely different the purification steps employed, the greater the degree of purification.

Ion exchange, dialysis, crystallization, electrophoresis, etc., are useful but cannot be discussed here. We will cover briefly only three techniques.

A Distillation

B Partition

C Adsorption

II THEORETICAL BACKGROUND

A Distillation

1 Raoult's Law states that the partial pressure of A(P_A) in an ideal mixture of volatile solvents is

P_A = mole fraction of A in liquid × vapor pressure of pure A at the temperature of the system.

The total vapor pressure (which, of course, sets the boiling point) is then $P_A + P_B + \ldots$. This is the basis for studies of distillation. The question of non-ideal solutions is too complicated to discuss. Most physical chemistry texts go into it at length.

2 Partially miscible liquids are a special case, often discussed as "steam distillation" although co-distillation is a better term. Quoting from Glasstone, "As long as the two layers are present, irrespective of their relative amounts, the total vapor pressure remains constant and the system boils at a definite temperature."

Besides the familiar case of steam distillation, non-polar organics can be co-distilled with polar liquids, e.g., insecticides with glycerol and polar compounds with non-polar solvents (i.e., mineral oil). The co-distillation can be done with superheated solvents, under vacuum. Where trace quantities of one component are involved, there may not be a two-phase system present and the solvent vapor serves only as a carrier gas. Here the solute can usually be recovered easily, however, so the method is worth consideration regardless of the mechanism.

In practical work, the three important points are the stability of the sample, its volatility, and the ease of recovery from the distillate.

3 Distillation from acid and basic solutions may also be useful. The principle behind this will be discussed in the solubility separation method.

B Partition

Partition is the principle utilized in liquid-liquid extraction, paper chromatography and gas chromatography. The fundamental law is the Distribution Law: "A dissolved substance distributes itself between the layers of a two-layer system so that at constant temperature the ratio of the concentrations is also constant". The Law is properly applied only to dilute solutions, but a first approximation to the distribution ratio can be obtained from the ratio of solubilities.

$$K = \frac{C_s}{C_w} \qquad K = \frac{S_s}{S_w}$$

K is a true constant but apparent discrepancies occur where the solute has different molecular weights in the two phases. Benzoic acid, for instance, is largely $\phi COOH$ plus some benzoate ion in water; in benzene it is the dimer $(\phi COOH)_2$. K then applies only to the species common to both layers, $\phi COOH$, and ignores the dimer.

Most texts on qualitative organic analysis contain a great deal of practical information on solubilities, while the usual undergraduate physical chemistry texts cover the essentials of the theory.

C Adsorption

There are two kinds of adsorption to be considered: physical (van der Waals) adsorption, and chemisorption. The theory of adsorption from solution is not as well understood as adsorption of gases but we can make a few comments.

Physical adsorption depends on the relatively weak van der Waals forces due to electrostatic attraction of dipoles, it being assumed that non-polar molecules act as oscillating dipoles; i.e., the nuclei of atoms and molecules form oscillating dipoles with the electrons. Since the bonds are weak, adsorption can be made reversible under proper conditions.

Chemisorption is thought of as actual chemical reaction, and the resulting bonds are much more difficult to break. Losses due to "irreversible adsorption" may therefore appear; this is particularly true of carbon, less so of silica and alumina.

III SOLUBILITY SEPARATION OF EXTRACTS

In the next section, we will describe a laboratory procedure which we have found very useful in handling carbon filter extracts. The extracts are split into acid, basic, and neutral fractions, and the neutral fraction is further separated by adsorption chromatography. This procedure has been extremely useful where the fractions are weighed and infrared spectra made. However, where volatile compounds are to be looked for by gas chromatography, it is almost imperative that a steam distillation step be included. Otherwise the mass of heavy, probably polymeric, material present causes much interference.

Really good clean-up methods are likely to be highly specific and need to be "tailor-made" to suit the situation. Since this lecture is concerned only with the "preliminary" purification, we have omitted a discussion of precise analytical partition and adsorption columns (thin-layer, ion exchange, paper and gas chromatography, gradient elution methods, etc.) Such methods constitute a second and higher degree of purification, but ordinarily require that they be preceded by a "rougher" primary purification.

IV NOTE ON LOSSES OF VOLATILE COMPOUNDS

It may not be generally recognized, but it is quite true that heavy losses of even moderately volatile compounds occur if one tries to evaporate off all of the solvent when preparing to weigh an extract. We have had losses of over 50% in attempting to carry out what we thought were very careful evaporations of ether from 10 to 20 mg amounts of phenols.

For this reason, we suggest that no extract or fraction thereof be evaporated down for weighing unless it is known to be completely non-volatile. This precaution becomes more necessary as the purity of the sample increases, because non-volatile impurities act as "solvents" to reduce the amount of loss (according to Raoult's Law).

REFERENCES

1 Maron and Prutton. Principles of Physical Chemistry (4th Edition) The Macmillan Co. 1965.

2 Moore. Physical Chemistry (3rd Ed.) Prentice Hall, Inc. 1965.

3 Glasstone and Lewis. Elements of Physical Chemistry. D. van Nostrand Co. 1960.

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LABORATORY PROCEDURES FOR THE PRELIMINARY SEPARATION OF EXTRACTS

I STEAM DISTILLATION OF EXTRACTS

Steam distillation need be only a very simple procedure. Put a gram or two of crude or partially purified sample in a small flask containing 50 - 100 ml of distilled water. Set up for ordinary distillation and boil off most of the water. Set up for ordinary distillation and boil off most of the water. If necessary, add more water to the flask and continue distilling until the distillate has no tract of cloudiness or a second phase as it drips from the condenser. Transfer the distillate to a separatory funnel, extract with a suitable solvent, and concentrate to a convenient volume. As the product is relatively volatile, do not try to evaporate to dryness if it can be avoided. The non-volatile residue in the distillation flask can also be recovered if necessary.

If it is desired to recover acids (e.g., phenols in the 4-AAP colorimetric method), make the solution acid before distilling; the distillate will then contain only neutral and acidic substances. The neutrals can then be separated during the extraction step. Basic materials remaining in the still pot, e.g., aniline, can then be recovered by making basic and distilling over a second fraction.

II SOLUBILITY SEPARATION OF EXTRACTS

A The organic contaminants from water as extracted from carbon or concentrated by liquid extraction ordinarily form an exceedingly complex mixture for which there is no one satisfactory separation procedure. If the analysis is directed towards a single component, the procedure may be designed for that purpose; otherwise a useful and generally applicable preliminary separation may be made on the basis of relative acidities.

B By extracting an ether solution of the sample with water, then with dilute hydrochloric acid, then sodium bicarbonate, and finally sodium hydroxide, a separation into water soluble, basic, strongly acidic, weakly acidic, and neutral fractions may be made. The portion insoluble in ether may also be recovered as an additional fraction. This method is obviously not suited to very volatile substances, nor to further separation of the water soluble fraction, nor to substances unstable in the presence of water or dilute acid or base; in addition, it must be remembered that substances whose partition coefficients are not extremely unfavorable to water may be present in several fractions in varying amounts.

C This procedure constitutes one of the best preliminary steps in analyzing any unknown sample and may often profitably be either preceded or followed by steam distillation (at various pH's) fractional crystallization, etc. Simple micro qualitative tests for nitrogen, halogens, sulfur, phosphorus, etc., are also often useful; for a more extended discussion, see the texts mentioned in the bibliography.

D General

The sample should be substantially free of solvent. The amount may vary considerably but one-half gram is a very convenient amount; as little as 100 mg or even less may be used, but in this case there may be large percentage errors. Ethyl ether has proven to be an excellent solvent although often not all the sample will dissolve in it; benzene or chloroform may be used although these solvents are more likely to cause troublesome emulsions.

III LABORATORY DIRECTIONS

A Solubility Separation Procedure

Solution in Ether

1 Weigh previously dried sample contained in a tared 50 ml beaker (approximately 0.5 gm preferred). Also, weigh a 125 ml flask for the Water Solubles (WS).

2 Dissolve the sample with a small amount of methanol (about 1 ml). Add the methanol dropwise and stir with a rigid wire until the sample reaches a syrupy consistency.

3 Add 30 ml ether to the sample. Not all will dissolve. Collect the Ether Insolubles (EI) on a sintered glass funnel with suction and set aside for paragraph 4. Pour the contents of the suction flask into a 125 ml separatory funnel equipped with a teflon stopcock. (Stopcock grease will contaminate the sample.)

Ether Insolubles (EI)

4 Dissolve "EI" previously collected on the sintered glass funnel with methanol. Wash funnel once with a small volume of chloroform and collect in the suction flask. Transfer filtrate to the original tared beaker with methanol. Evaporate to dryness on a steam bath and record weight for Ether Insolubles.

Separation of the Water Solubles (WS)

5 Shake the ether solution(3) three times with 15 ml portions of distilled water. Drain the water layers into the weighed "WS" flask after each shaking. Evaporate the Water Soluble fraction to dryness on a steam bath with a jet of clean, dry air and record weight for Water Solubles.

Separation of Amine Fraction (B)

6 Mark flask "HCl". Shake ether solution three times with 15 ml portions of 5% HCl and drain aqueous layers into HCl flask after each shaking. Make this aqueous solution strongly basic by carefully adding NaOH pellets (about 30) or 25% NaOH. The solution becomes darker at this point. Set aside for back-extraction.

Separation of Strong Acid Fraction (SA)

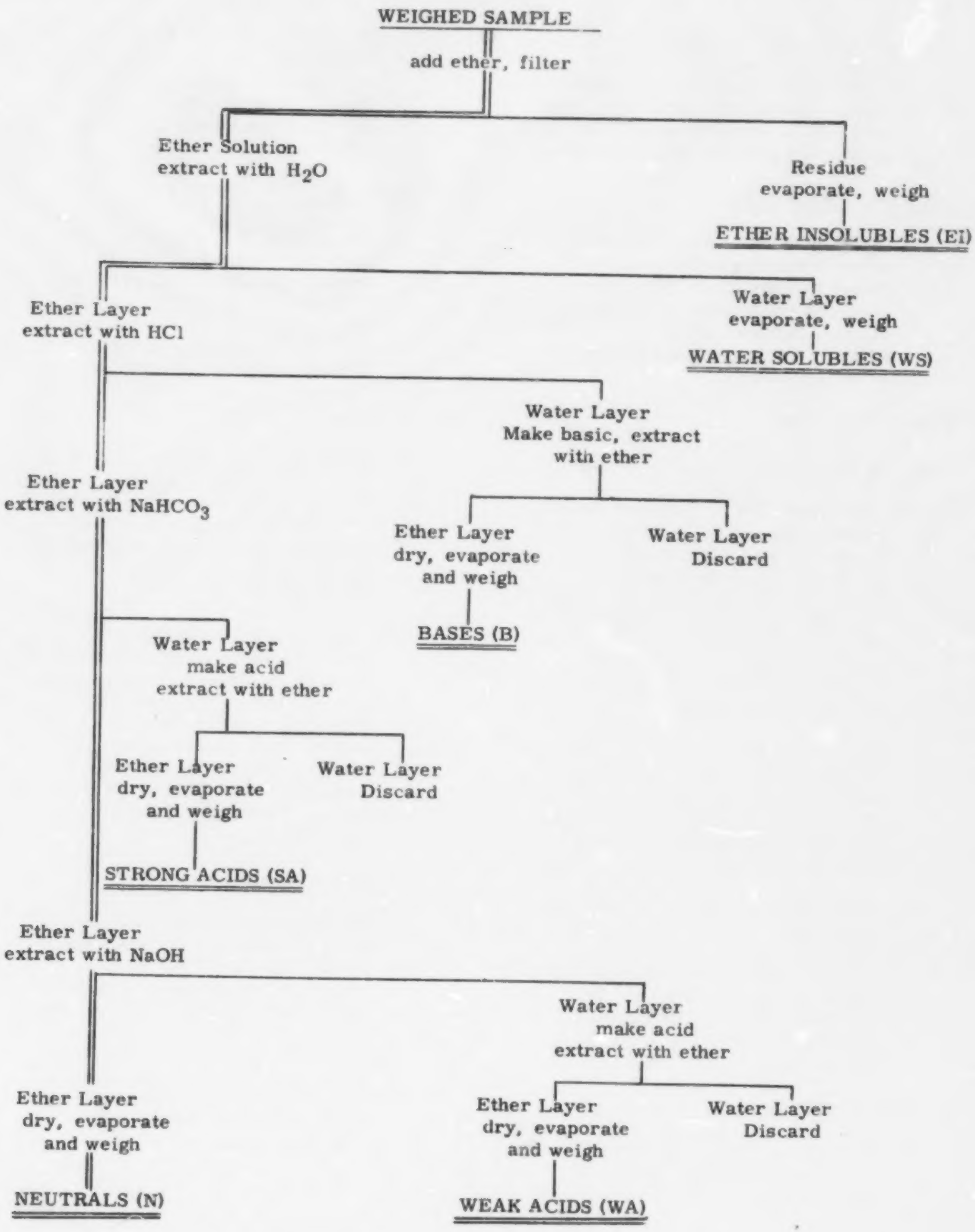
7 Restore, original ether volume to 30 ml, if necessary. Mark flask "$NaHCO_3$". Shake three times with 15 ml portions of 5% $NaHCO_3$ and drain aqueous layers into $NaHCO_3$ flask after each shaking. Acidify aqueous solution in flask by adding sufficient concentrated HCl (dropwise because of liberation of CO_2) until strongly acid to litmus paper. About 4 ml is sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Separation of Weak Acid Fraction (WA)

8 Restore original ether volume to 30 ml, if necessary. Mark flask "NaOH". Shake three times with 15 ml portions of 5% NaOH and drain aqueous layers into "NaOH" flask after each shaking. Wash once with 15 ml distilled water and drain this into "NaOH" flask.

CAUTION: Tilt separatory funnel gently a few times, instead of shaking the first portion, as emulsions often occur.

If a small amount of the water wash remains emulsified, after draining, add several drops of saturated Na_2SO_4 solution and shake. This may help to break


WEIGHED SAMPLE
add ether, filter
Ether Solution
extract with H_2O
Residue
evaporate, weigh
ETHER INSOLUBLES (EI)
Ether Layer
extract with HCl
Water Layer
evaporate, weigh
WATER SOLUBLES (WS)
Water Layer
Make basic, extract
with ether
Ether Layer
extract with $NaHCO_3$
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
BASES (B)
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
STRONG ACIDS (SA)
Ether Layer
extract with NaOH
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
Ether Layer
dry, evaporate
and weigh
NEUTRALS (N)
WEAK ACIDS (WA)

the emulsion. Acidify aqueous solution in flask with concentrated HCl until strongly acid to litmus paper. About 8 ml sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Neutral Fraction (N)

9 Mark flask "N". Pour the ether layer remaining in the separatory funnel into the flask "N". Add about 10 gm of anhydrous sodium sulfate, cap with aluminum foil and allow to stand overnight to dry the ether solution.

B Back Extraction

Strong Acids (SA)

10 Mark flask "SA". Transfer $NaHCO_3$ extract from step 7 to separatory funnel and shake several times, carefully, relieving pressure caused by evolution of CO_2. Shake the $NaHCO_3$ extract, previously made acid, with a 15 ml portion of ether. Caution: Release CO_2 during first shaking repeatedly to avoid pressure build-up.

Drain the aqueous layer into the $NaHCO_3$ flask. Pour the ether layer into the "SA" flask. Return aqueous layer to separatory funnel and repeat extraction two more times with 15 ml portions of ether.

After all the ether layers have been collected in the "SA" flask, add about 10 gms anhydrous sodium sulfate, cap with foil and allow to stand overnight to dry the ether solution. Discard the aqueous $NaHCO_3$ portion which should be almost colorless.

Weak Acids (WA)

11 Mark flask "WA". Shake the NaOH extract, previously made acid, three times with 15 ml portions of ether and collect in flask "WA" as in step 10. Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous NaOH portion which should be almost colorless.

Bases (B)

12 Mark flask "B". Shake the HCl extract, previously made basic, three times with 15 ml portions of ether and collect in flask "B". Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous HCl portion.

C Transfer

13 Weigh four flasks and mark them "N", "WA", "SA", and "B". Record weights of each. Transfer the corresponding dried ether solutions from the Na_2SO_4 into the weighed flasks by filtering through filter paper.

14 Evaporate the ether from the above fractions with the aid of clean, dry circulating air and gentle heat on a steam bath. The ether solutions should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components. Record the weight of the dried fractions.

D Chromatographic Separation

15 Weigh a 10 ml beaker and record weight. Dissolve the Neutrals in about 5 ml anhydrous ether, transfer most of the Neutrals to the beaker and place the remaining few drops in a vial for infrared analysis. Wash the (N) flask with ether and pour washings into vial also. Dry the Neutrals contained in the beaker by spontaneous evaporation and record weight.

16 Weigh a set of 125 ml Erlenmeyer flasks and mark them Aliphatics, Aromatics and Oxys. Record weights of each.

17 Fill the chromatographic column with 4 1/2" of silica gel and tap down to about 4". Place the Aliphatics flask under the column. Add about 20 ml of iso-octane to wet the column.

Aliphatics

18 Before the iso-octane reaches the level of the silica gel, add the Neutrals which have been previously adsorbed onto a small amount of silica gel by stirring in the beaker with a rigid wire. Elute with 85 ml iso-octane. Rinse the beaker with a small portion and add to the column first. Allow this volume to enter silica gel before adding the remainder of the iso-octane. Collect the eluent in the Aliphatics flask, evaporate to dryness with the aid of gentle heat and circulating air.

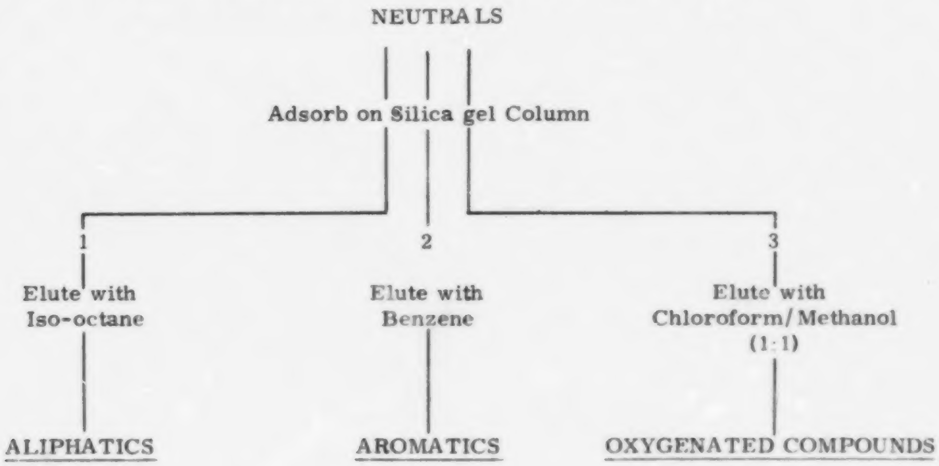
Aromatics

19 Place the Aromatics flask under the column. Elute column with 85 ml of Benzene-rinsing the beaker with a small portion and adding immediately as the previous eluent reaches the level of the silica gel. Allow this volume to enter silica gel before adding remainder of benzene. Evaporate with the aid of gentle heat and circulating air. The Aromatics fraction should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components.

Oxys

20 Place the Oxys flask under the column. Elute with 85 ml of a 1:1 methanol chloroform mixture - rinsing the beaker with a small portion and proceed as before. Evaporate to dryness with the aid of gentle heat and circulating air.

CHROMATOGRAPHIC SEPARATION OF NEUTRALS



21 Record weights of the dried Aliphatics, Aromatics and Oxys.

NOTE: (All chromatographic solvents should be redistilled before use)

REFERENCES

1 Shriner, Fuson and Curtin. The Systematic Identification of Organic Compounds. 4th ed. John Wiley & Sons. New York. 1956.

2 Cheronis and Entrikin. Semimicro Qualitative Organic Analysis. Thomas Y. Crowell Co. New York. 1947.

3 Cheronis. Micro and Semimicro Methods. Vol. VI of the series Techniques of Organic Chemistry. Arnold Weissberger, Ed. Interscience. New York. 1954.

4 Schneider. Qualitative Organic Microanalysis. John Wiley & Sons. New York. 1946.

These books deal principally with chemical methods of identification; Shriner, Fuson and Curtin and Cheronis and Entrikin are recommended for those new to the field. For instrumental analysis and quantitative work, see standard works in those fields.

This outline was prepared by R. H. Burttschell, former Chemist, Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, 4676 Columbia Parkway, Cincinnati, OH 45226, and J. J. Lichtenberg, Chief, Organic Analyses Unit, Analytical Quality Control Laboratory, FWQA, 1014 Broadway, Cincinnati, OH 45202

LIQUID - LIQUID EXTRACTION METHODS FOR RECOVERY OF ORGANIC SUBSTANCES FROM STREAMS

I GENERAL PRINCIPLES

Liquid extraction is familiar to all chemists in the form of separatory funnel operations. A water solution of some organic substance is shaken with ether, the two liquid layers are allowed to separate, the water layer is discarded and the desired organic is recovered in the ether layer. A transfer of material has been accomplished from one liquid phase to another. This transfer is not instantaneous. The two liquids are brought into close contact by shaking actively for several minutes, until equilibrium is reached.

The condition at equilibrium is determined bv a property of the solute, the partition coefficient:

$$K = \frac{C_s}{C_w}$$

Where: K is the partition coefficient

C_s is the concentration in the extracting solvent

C_w is the concentration in water

In the ideal situation, the value of K is fixed whether both liquids are saturated or are extremely dilute (for any given pair of solvents at a fixed temperature).

The portion of the solute transferred depends also on the relative volumes of the two liquids.

$$\frac{\text{Solute in organic layer}}{\text{Solute in water}} = \frac{C_s}{C_w}\,\frac{V_s}{V_w} = K\,\frac{V_s}{V_w}$$

Where: V_s is the volume of extracting solvent

V_w is the volume of water

Obviously, $C_sV_s + C_wV_w$ is the total weight of solute.

II BATCH EXTRACTION - SINGLE STAGE

Consider an ester with a partition coefficient: K = 1000 between hexane and water.

Now let us extract 20 liters of water containing 1 ppm ester with 100 ml of hexane.

$$\frac{\text{Solute in hexane}}{\text{Solute in } H_2O} = K\,\frac{V_s}{V_w} = 1000 \times \frac{100}{20,000} = \frac{5}{1} \text{ for an 83\% recovery.}$$

Batch extraction can be extremely useful in cases where the partition coefficient is very favorable. The following example deals with parathion with a K value assumed to be approximately 2000 between hexane and water.

If we extract one liter of water saturated with the insecticide, using 100 ml of hexane, then:

$$\frac{\text{Parathion in hexane}}{\text{Parathion in water}} = 2,000 \times \frac{100}{1000} = \frac{200}{1}$$

which is essentially quantitative.

Many chlorinated insecticides have partition coefficients even more favorable than in the example above. Since there are specific analytical methods of high sensitivity for many of these insecticides, it is practical to use batch extraction to prepare the sample for analysis. Depending on the analytical procedure and the concentration, water samples ranging from a liter to five gallons have been employed. Securing equilibrium is the main problem.

The equipment required for batch liquid extraction is quite simple. In many cases, separatory funnels ranging in capacity up to five liters may be used. Modifications may be devised for larger samples. A convenient device is a five-gallon carboy, using a solvent heavier than water, such as chloroform. Contact between the two liquids is accomplished by vigorous mechanical stirring, then the two phases are allowed to separate and the water is removed by decantation. The chloroform is transferred to a separatory funnel, where the removal of water is completed.

Increased extraction efficiency can be attained by performing multiple extractions of the sample with successive lots of solvent.

III BATCH EXTRACTION - CONTINUOUS

When the partition coefficient is not very favorable, extraction efficiency can be increased by using larger volumes of extraction solvent. One procedure was described in the preceding section. The continuous extractor is a device to accomplish the same result while limiting the solvent to a convenient volume.

In the continuous extractor, a relatively small volume of solvent circulates first through the water sample, where the extraction occurs, then to a distilling flask where pure solvent is distilled and passes back to the extractor. The extract concentrates in the distilling flask. Thus, it is possible to carry out the extraction with what in effect is a large volume of solvent and yet concentrate the extract in only a little solvent. It is evident that for this method the extract must be much less volatile than the solvent, or it will circulate, too.

Batch continuous extraction is rarely useful in water pollution studies, because the amount of water sample that it can be applied to is usually much too small. But the equipment can be operated for long periods with little attention and thus has been used to perform exhaustive extractions on a small scale for specialized analyses, e.g., with sewage.

A large device for several stages of extraction and up to 135 liters has been described by Kahn & Wayman; see Anal. Chem. 36, 1340. (1964).

The counter-current extraction system greatly amplifies the advantages of multiple stage extraction. We have used an all-glass device of this type for 5 to 20 gallon volumes of waste and it is highly efficient. However, it is fragile and requires a good deal of space, solvent, and attention.

IV MISCELLANEOUS METHODS

Recent work in our laboratories has shown that reverse-phase partition columns are quite suitable for extracting fairly large volumes of water provided that the solute and the organic solvent are relatively insoluble in water.

The partition column uses a support such as silica or a diatomaceous earth to hold an aqueous (or other strongly polar) solvent. A non-polar solvent is then allowed to percolate slowly down the column while a continuous partition of the solute takes place between the phases. With the reverse-phase column, the stationary support is rendered hydrophobic with silicone, etc. and serves to hold the non-polar organic phase in place while the aqueous phase flows downward through it.

Another device for 10-20 liter quantities is a carboy containing the desired amounts of water and solvent. It can be rolled or shaken until equilibrated, then the solvent removed by syphoning. The difficulty, of course, is in obtaining equilibrium.

This outline was prepared by R. H. Burttschell, former Chemist, Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, R. A. Taft Water Research Center.

THIN-LAYER CHROMATOGRAPHY

I INTRODUCTION

A Historical

1 Adsorption chromatography was discovered by Tswett in 1903. Tswett found that plant pigments (e.g., chlorophylls) can be separated by filtering a solution of them in petroleum ether through a column of calcium carbonate. He noticed that yellow and green zones were formed in the column.

2 Adsorption chromatography was introduced in 1931 when the method was used for the preparative chemistry of the polyene pigments, for example the separation of carotene into its components.

3 Partition chromatography for hydrophilic compounds was introduced in 1941.

4 Thin-layer chromatography was introduced by Stahl in 1956. He described an ingenious and practicable device for preparing layers of silica gel about 250 microns thick with a plaster of paris binder.

5 Thin-layer chromatography has proven useful for the quantitative estimation of pesticides and for the "clean-up" of sample aliquots prior to the estimation of their components by gas chromatography.

B Theory

1 Thin-layer chromatography is an analytical technique which has proven useful for the separation of components in a given system and to evaluate the components in that system.

2 All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in equilibrium with a stationary phase. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called adsorption chromatography.

3 When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be partition chromatography.

4 Diagnostic or qualitative thin-layer chromatography is used to determine the number of components in a system.

5 Preparative thin-layer chromatography is used to separate a mixture into its components in such a way that reasonable amounts can be isolated and studied.

6 Quantitative thin-layer chromatography is used to measure the amount of the components present. This is done by estimating the density of the spots as compared to standard spots.

II DEFINITION OF TERMS

A Adsorbent. The finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography.

B Layer. The thin layer of adsorbent bound or unbound, deposited on a glass plate.

C Spotting. The application of the substance to the thin layer adsorbent.

D Development. The passing of a liquid through the layer to effect a separation.

E Developer. The liquid used for the development.

F Eluent. The complete removal of a substance from the adsorbent layer.

G Visualization. The rendering visible of the results of a developed chromatogram.

H Ratio facter "R_f". The distance traveled by a given substance divided by the distance traveled by the solvent front. Both measured as the origin.

III ADSORBENTS

A Silicic Acid or Silica Gel

Chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

B Aluminum Oxide or Alumina

C Diatomaceous Earth

D Powdered Cellulose

E Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

IV PREPARATION OF PLATES

A Methods

1 Spreading or spraying of slurries

2 Dipping in a slurry

B Equipment

1 Kirchner type

2 Stahl type

3 Microchromatoplates

C Layer thickness

1 For qualitative analysis thin layers (250 microns) are used.

2 For preparative thin layer thicker layers may be used.

D Moisture Content

1 Trace quantities of water greatly reduce the activity of various adsorbents.

2 Normally plates are activated by drying at 110°C for one hour and then stored in a desiccator.

V TECHNIQUE

A Spotting the Plate

1 The activity of the layer is affected by humidity and temperature.

2 The sample is applied so that the direction of the development is opposite to the direction in which the layer has been applied.

3 Use of spotting template

4 Use of micro-pipette and micro-liter syringe

5 Size of sample spots

6 Use of standards

B Solvent System

1 Investigation of single and multi-component systems

2 Carbon tetrachloride, hexane, and n-heptane as solvents for separation of pesticides

C Development of the Plate

1 Development tank

2 The plate is placed in a glass tank containing the solvent. When the solvent front reaches the upper reference line (10 cm) the plate is removed and the solvent is allowed to evaporate.

VI VISUALIZATION

A Rhodamine B

1 Spray reagents - Rhodamine B, spirit soluble, 0.1 mg per ml in ethanol

2 Method of spraying

3 Advantages of Rhodamine B

a The pesticide is not destroyed.

b The exact position of the pesticide can be determined.

c The use of a selective solvent permits the pesticide to be eluted from the adsorbent while the Rhodamine B is retained.

B Impregnation of Plates with $AgNO_3$

1 Increased sensitivity for quantitative estimation

2 Elimination of spraying

C Exposure to UV Light

1 Pesticides appear as quenched areas in the fluorescent background.

2 Recovery studies using Rhodamine B spray are comparable to those using silver nitrate spray. The following pesticides may be seen at the 10 μg level under UV light after Rhodamine B spray:

endrin
dieldrin
DDT
aldrin
lindane
chlordane
parathion
methyl parathion
heptachlor
heptachlor epoxide
methoxychlor
toxaphene
DDE
ovex
tedion

D Suggested systems for adsorption chromatography are given in Table 1.

E Recovery

1 Elution of pesticides from the thin layer with a mixture of ethyl and petroleum ethers.

2 Recovery zones as shown in Figure 1.

3 Silica gel collection assembly - Figure 2.

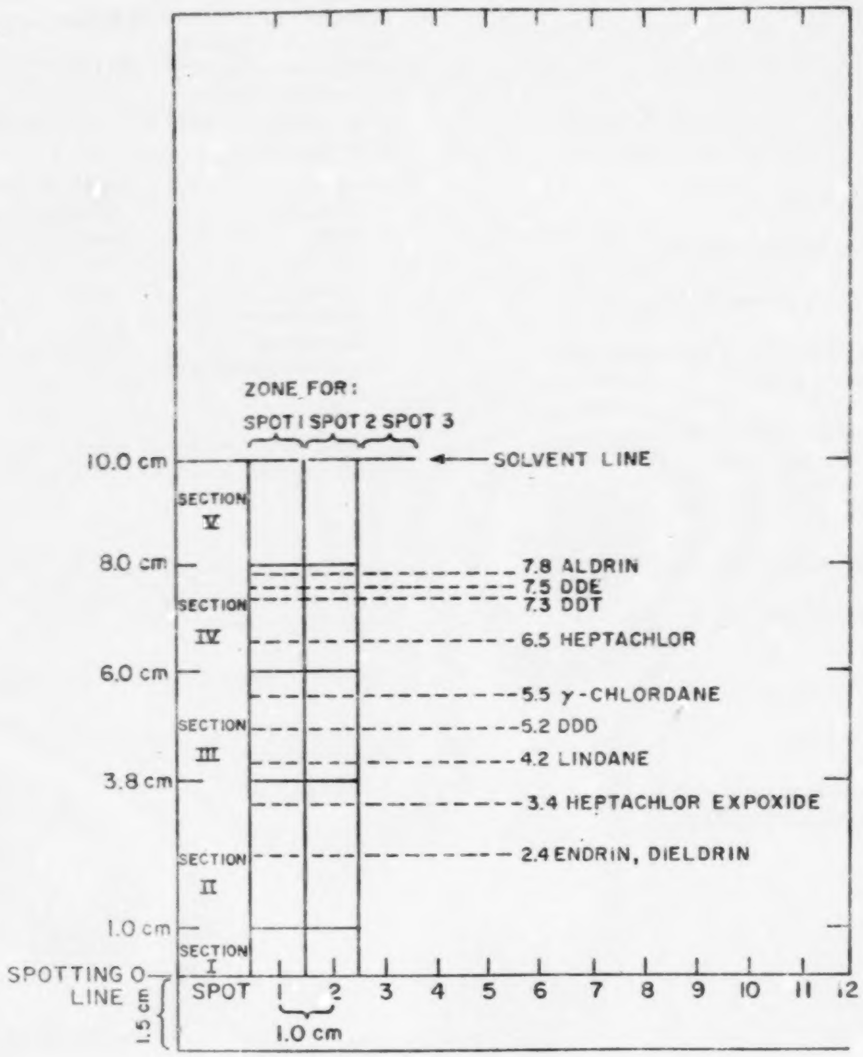
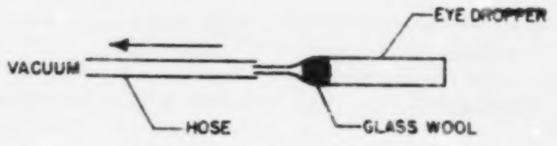

ZONE FOR:
SPOT 1
SPOT 2
SPOT 3
SOLVENT LINE
10.0 cm
SECTION V
8.0 cm
7.8 ALDRIN
7.5 DDE
7.3 DDT
SECTION IV
6.5 HEPTACHLOR
6.0 cm
5.5 γ-CHLORDANE
SECTION III
5.2 DDD
4.2 LINDANE
3.8 cm
3.4 HEPTACHLOR EXPOXIDE
SECTION II
2.4 ENDRIN, DIELDRIN
1.0 cm
SECTION I
SPOTTING 0
LINE
1.5 cm
SPOT 1 2 3 4 5 6 7 8 9 10 11 12
1.0 cm

Figure 1. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers.



SILICA GEL COLLECTION ASSEMBLY

FIGURE 2

REFERENCES

1 Smith, D. and Eichelberger, J. Thin-Layer Chromatography of Carbon Adsorption Extracts Prior to Gas Chromatographic Analysis for Pesticides. J. Water Poll. Control Fed. 37, 77, (1965).

2 Damaska, W. H. F.D.A. Cincinnati District, Private Communication. October, 1964.

3 Randerath, Kurt. Thin-Layer Chromatography, Academic Press. New York. 1963.

4 Bobbitt, J. M. Thin-Layer Chromatography. Theinhold, New York. 1963.

This outline was prepared by P.F. Hallbach, Chemist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

TABLE 1

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
1. Long-chain (over eight carbons) aliphatic ketones	silica gel G	a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures	phosphomolybdic acid (2, 4-DNPH)
2. Aliphatic and aromatic aldehydes and ketones	aluminum oxide	a. benzene b. benzene-EtOH (98:2) (95:5) (90:10) c. $CHCl_3$ d. EtOEt e. pet. ether- benzene (1:1)	2, 4-DNPH
3. Aromatic aldehydes and ketones	silica gel G	hexane-EtOAc (4:1) (3:2)	(U. V. on phosphors)
4. Vanillin and other substituted benzaldehydes	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. $CHCl_3$-EtOAc (98:2) d. decalin-CH_2Cl_2-MeOH (5:4:1)	(U. V. on phosphors)
5. 2, 4-Dinitrophenylhydrazones of aldehydes	silica gel G	a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic	colored
6. Miscellaneous alkaloids	silica gel G	a. $CHCl_3$-acetone-diethylamine (5:4:1) b. $CHCl_3$-diethylamine (9:1) c. cyclohexane-$CHCl_3$-diethylamine (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1)	(Dragendorff's Reagent)
	aluminum oxide	f. $CHCl_3$ g. cyclohexane-$CHCl_3$ (3:7) plus 0. 05% diethylamine	
7. Alkaloids and barbiturates in toxicology	silica gel G	a. MeOH b. $CHCl_3$-EtOEt (85:15)	(Dragendorff's Reagent)
8. Strong-base amines	unbound aluminum oxide	a. acetone-heptane (1:1) b. $CHCl_3/NH_3$ (sat. at 22°)-EtOH (96%) (30:1)	I_2 vapor-U. V. (Dragendorff's Reagent)
9. Sugar acetates and inositol acetates	silica gel G	benzene with 2-10% MeOH	H_2O

T..BLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
10. Aldose 2,4-DNPH's	a. aluminum oxide G b. silica gel G	a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1)	a. colored compounds b. NaOH
11. Cardiac glycosides	silica gel G	a. $CHCl_3$-MeOH (9:1) b. $CHCl_3$-acetone (65:35)	H_2SO_4 and heat
12. Dicarboxylic acids	silica gel G	a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4)	bromphenol blue acidified with citric acid
13. p-Hydroxybenzoic acid esters	silica gel G	pentane-HOAc (88:12)	(U.V. on phosphors)
14. Sulfonamides	silica gel G	$CHCl_3$-EtOH- heptane (1:1:1)	p-dimethylamino-benzaldehyde, acidified (U.V. on phosphors)
15. Food dyes	silica gel G	a. $CHCl_3$-acetic anhydride (75:2) b. benzene c. methyl ethyl ketone-HOAc-MeOH (40:5:5)	$SbCl_3$ in $CHCl_3$
16. Misc. essential oils	silica gel G	benzene-$CHCl_3$ (1:1)	$SbCl_3$ in $CHCl_3$
17. Coumarins	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc ([illegible]:2)	(U.V. on phosphors)
18. Alkali metals-Na^{1+}, Li^{1+}, K^{1+}, Mg^{2+}	purified silica gel G	EtOH-HOAc (100:1)	acid violet (1.5% aqueous soln.)
19. 24 ferrocene	silica gel G	a. benzene b. benzene-EtOH (30:1) (15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1)	(U.V. on phosphors)
20. Misc. lipids	silica gel G	a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98.5:1.5)	(H_2SO_4 and heat)
21. Fatty acid methyl esters	silica gel G	hexane-EtOEt mixtures (up to 30% EtOEt)	a. I_2 b. H_2SO_4 and heat
22. Glycerides	silica gel G	a. Skellysolve F-EtOEt (70:30)	H_2SO_4 and heat

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

	Compounds	Adsorbent	Developer	Visualization
			b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40) (35:65) (85:15)	
23.	Long-chain aliphatic alcohols	silica gel G	a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90) (70:30)	H_2SO_4 and heat
24.	Phenols	a. starch-bound silicic acid kieselguhr (1:1)	a. xylene b. xylene-$CHCl_3$ (3:1) (1:1) (1:3) c. $CHCl_3$	(U.V. on phosphors)
25.	Phenols and phenolic acids	starch-bound silicic acid with phosphors	a. Skellysolve B- EtOEt (3:7) b. Skellysolve B- EtOAc (1:3) c. Skellysolve B-acetone (3:1)	(U.V. on phosphors)
26.	Phenols	plaster-of-Paris bound silica gel	a. hexane-EtOAc (4:1) (3:2) b. benzene-EtOEt (4:1) c. benzene	(U.V. on phosphors)
27.	3,5-Dinitrobenzoates of alcohols and phenols	silica gel G	a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15) (75:25) c. toluene-EtOAc (90:10)	colored compounds
28.	Steroids	silica gel G	a. benzene b. benzene-EtOAc (9:1 (2:1) c. cyclohexane-EtOAc (9:1) (19:1) d. 1,2-dichloroethane	$SbCl_3$ in $CHCl_3$
29.	19-Nor-steroids	silica gel G	EtOAc-cyclohexane mixtures	$SbCl_3$ in $CHCl_3$
30.	Blood cholesterol and cholesterol esters	silica gel G	a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. $CHCl_3$	$SbCl_3$ in $CHCl_3$
31.	Triterpenoids	silica gel G	a. isopropyl ether-acetone (5:2) (19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH_2Cl_2	(H_2SO_4 and heat)

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
32. Carotenes and fat-soluble vitamins, A, D, E, K	unbound aluminum oxide	a. MeOH b. CCl_4 c. xylene	H_2SO_4 and heat
33. Thiophene derivatives	a. silica gel G b. aluminum oxide G	a. benzene-$CHCl_3$ (9:1) b. MeOH c. pet. ether	(U.V. on phosphors)
34. Plasticizers (phthalates, phosphates and other esters)	silica gel G with phosphors	a. isooctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether - hexane (80:20)	a. (U.V. on phosphors) b. I_2

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)

2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104° C) and 4-Methylcyclohexane (B.P. 103° C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8° C) and Benzene (B.P. 80.2° C)	Chromosorb Newsletter (FF-104)

C Disadvantages

1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.

2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector.(See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

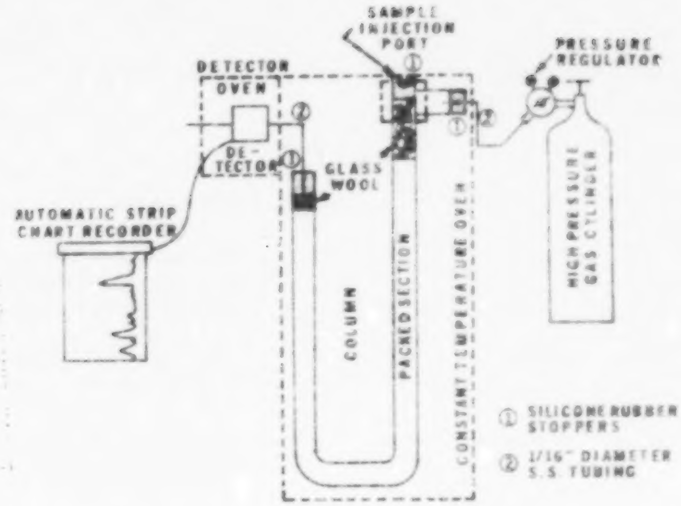

SAMPLE INJECTION PORT
PRESSURE REGULATOR
DETECTOR OVEN
DE-TECTOR
GLASS WOOL
AUTOMATIC STRIP CHART RECORDER
COLUMN
PACKED SECTION
CONSTANT TEMPERATURE OVEN
HIGH PRESSURE GAS CYLINDER
① SILICONE RUBBER STOPPERS
② 1/16" DIAMETER S.S. TUBING

Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH*

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m^2/gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B. P. 80.1°C) and cyclohexane (B. P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

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*Reproduced (with permission) from Chemistry. (37:11, p. 13. November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (picogram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture,

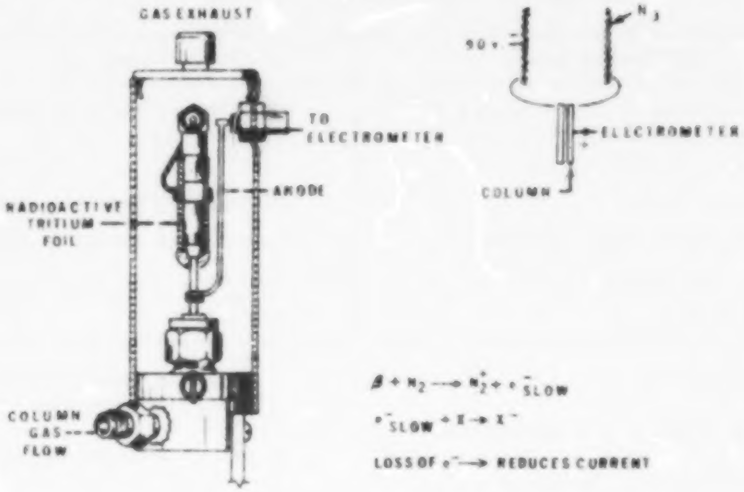


Figure 2. ELECTRON CAPTURE DETECTOR (Wilkens Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO_2, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO_2 produced is continuously titrated by I_2 which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH_3 which is then titrated by H^+ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds.

The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

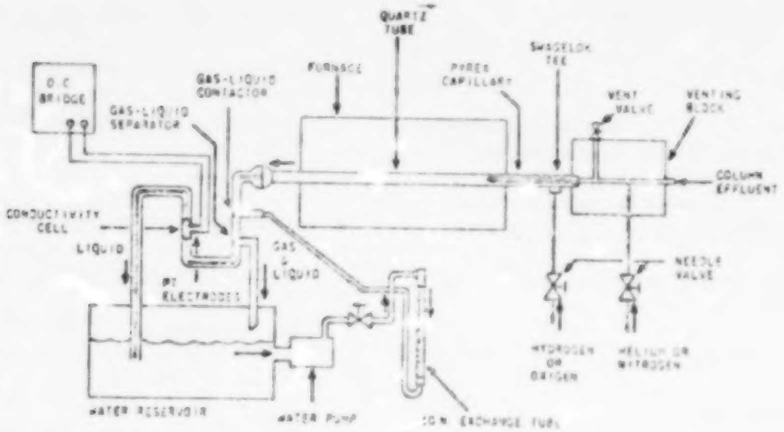

D.C. BRIDGE
GAS-LIQUID SEPARATOR
GAS-LIQUID CONTACTOR
FURNACE
QUARTZ TUBE
PYREX CAPILLARY
SWAGELOK TEE
VENT VALVE
VENTING BLOCK
COLUMN EFFLUENT
CONDUCTIVITY CELL
LIQUID
PT ELECTRODES
GAS & LIQUID
NEEDLE VALVE
HYDROGEN OR OXYGEN
HELIUM OR NITROGEN
WATER RESERVOIR
WATER PUMP
ION EXCHANGE TUBE

Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR (Coulson Instruments Company)

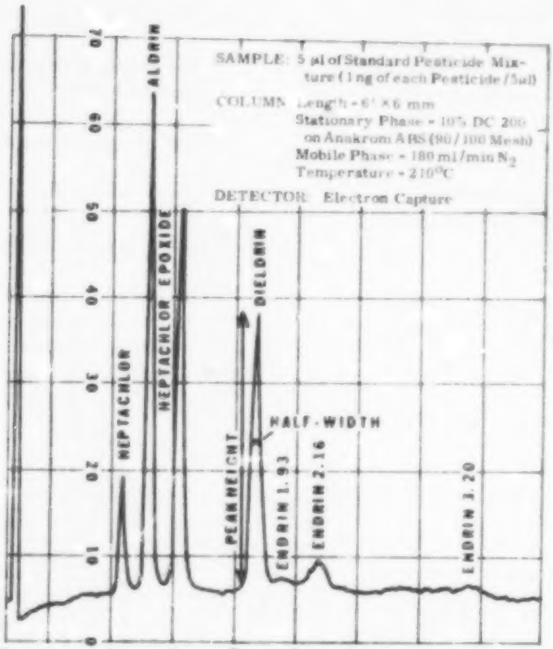


Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

1 Physical parameters of column operation

a Column length

b Column temperature

c Carrier gas flow rate

2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME } (R_V) = \text{RETENTION TIME } (R_T) \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

1 Planimeter

2 Triangulation

3 Peak height × half-width (see dieldrin peak in Figure 3)

AREA = peak height × peak half-width

4 Disc integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R_T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

1 A steady basline is obtained.

2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

BOOKS

1 Dal Nogare, S. and Juvet, R.S., Jr. Gas-Liquid Chromatography. New York: Interscience. 1962.

2 Littlewood, A.B. Gas Chromatography. New York: Academic Press. 1962.

NEWSLETTERS

1 Aerograph Gas Chromatography Newsletter. Wilkens Instrument and Research, Inc., P.O. Box 313 Walnut Creek, California.

2 F & M Gas Chromatography Newsleter. F & M Scientific Corporation. Starr Road and Route 41, Avondale, Pa.

3 Gas-Chrom Newsletter. Applied Science Laboratory, Inc. State College, Pa. 16801.

This outline was prepared by B. A. Punghorst, Chemist, formerly with FWQA National Training Center, Cincinnati, OH 45226

BASIC THEORY OF GAS-LIQUID CHROMATOGRAPHY

(PART II)

Gas chromatography is a method for separating the components of a mixture of compounds of sufficient volatility to be varporized. It is usually used to identify and quantitate the components in a mixture; it also is used to prepare small amounts of extremely pure materials. For this reason it is frequently used in conjunction with mass spectrometry, infrared spectroscopy, or NMR spectroscopy.

In chromatography the stationary phase may be a gas, liquid, or a solid, and the mobile phase likewise. The various possible combinations are shown in Table 1.

Table 1.

		Stationary Phase		
		gas	liquid	solid
Mobile Phase	gas	---	GC	GS
	liquid	---	LL	LS
	solid	---	---	zone refining

The apparatus used consists of a long, thin column packed with a solid support, such as firebrick crushed and screened, coated with a liquid phase, such as stopcock grease, through which is passing a carrier gas, frequently helium or nitrogen. Ahead of the column is the injector, through which the sample is introduced, and at the exit is the detector, which produces an electrical signal when the sample is emerging from the column. This signal is fed to a strip chart recorder. The heart of the system is the column, the part in which the separation takes place.

Henry's law states that a solute will distribute itself between the gas and liquid phases. That is, an equilibrium is established in which part of the solute is in the liquid phase and part is in the gas phase. This condition is defined by the Henry's Law Constant, or

$$\text{Partition Coefficient, } K = \frac{\text{amt. in liquid phase}}{\text{amt. in gas phase}}.$$

Different substances have different partition coefficients. A substance will only move down the column when it is in the gas phase. Thus a substance which spends the largest percentage of its time in the gas phase will come out of the column, or elute, first.

Imagine that a column is made up of a series of buckets partially filled with a stationary liquid phase. A unit amount of a substance to be separated is introduced into the first bucket and is allowed to distribute itself between the liquid and gas phases within the bucket until an equilibrium has been established. Imagine that the substance in the gas phase is then swept into a second bucket by a carrier gas and that both buckets are allowed to come to a new equilibrium.

This process is repeated with a 3rd, 4th, and 5th bucket as illustrated in Figure 1. The column can be considered as consisting of thousands of these buckets. It is seen that the substance distributes itself along the column as a Gaussian function and moves along the column as a function of time.

In an ideal column the profile would be as shown in Figure 2, with the material in the gas phase moving down the column and entering the liquid phase at the leading edge while it is coming from the liquid into the gas phase at the trailing edge.

Equilibrium 1.

$\frac{1/2}{1/2}$

Step 1.

$\frac{\ }{1/2}$ 1/2 $\frac{\ }{\ }$

Equilibrium 2.

$\frac{1/4}{1/4}$ $\frac{1/4}{1/4}$

Step 2.

$\frac{\ }{1/4}$ 1/4 $\frac{\ }{1/4}$ 1/4 $\frac{\ }{\ }$

Equilibrium 3.

$\frac{1/8}{1/8}$ $\frac{1/4}{1/4}$ $\frac{1/8}{1/8}$

Step 3.

$\frac{\ }{1/8}$ 1/8 $\frac{\ }{1/4}$ 1/4 $\frac{\ }{1/8}$ 1/8 $\frac{\ }{\ }$

Equilibrium 4.

$\frac{1/16}{1/16}$ $\frac{3/16}{3/16}$ $\frac{3/16}{3/16}$ $\frac{1/16}{1/16}$

Step 4.

$\frac{\ }{1/16}$ 1/16 $\frac{\ }{3/16}$ 3/16 $\frac{\ }{3/16}$ 3/16 $\frac{\ }{1/16}$ 1/16 $\frac{\ }{\ }$

Equilibrium 5.

$\frac{1/32}{1/32}$ $\frac{1/8}{1/8}$ $\frac{3/16}{3/16}$ $\frac{1/8}{1/8}$ $\frac{1/32}{1/32}$

Figure 1. DISTRIBUTION OF A SUBSTANCE ALONG A GAS CHROMATOGRAPHIC COLUMN

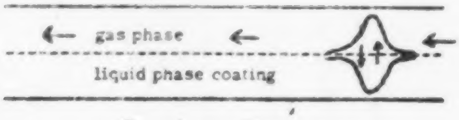


Figure 2.

Since separation takes place because of differences in K, the stationary liquid phase should dissolve all components to some extent, but no two to exactly the same extent. Since these differences may be very small, columns are usually of some length and must be very efficient. The same argument follows when solid adsorbants are used instead of liquid phases, as in gas-solid chromatography.

Efficiency is the ability to separate two substances and is defined in several ways. Let us examine a typical simple chromatogram and define some terms. Figure 3 shows the separation of a mixture of cyclohexane, benzene, and toluene. The point of injection is marked "start". This is followed by the air peak, which is a measure of the dead volume of the column. By drawing tangents to the peaks we determine the center. The distance from start to the center of the peak is the retention time, since the recorder is running at constant speed, and is designated by t_R. The peak width, w, is the intersection of the tangents with the base line.

The adjusted retention time, t_R, is the retention time minus the distance from the start to the air peak. The retention volume, V_R, is the retention time, t_R, times the carrier gas flow rate, F_c. Likewise, the adjusted retention volume $V'_R = t'_R\,F_c$. We speak of the number of plates, n, in a column. This is calculated from the expression, $n = 16\frac{(t_R)}{w}$. The height equivalent to a theoretical plate, HETP, is the column length in mm divided by the number of plates, n. For Benzene, in Figure 3, $w_1 = 9$ mm, $t_1 = 56$ mm, $n = 16(\frac{56}{9})^2 = 620$ plates. Note that the dimensions cancel. HETP $= \frac{5 \times 12 \times 25}{620}$ mm = 2.4 mm. It is the HETP, or plate height that is commonly used to express column performance.

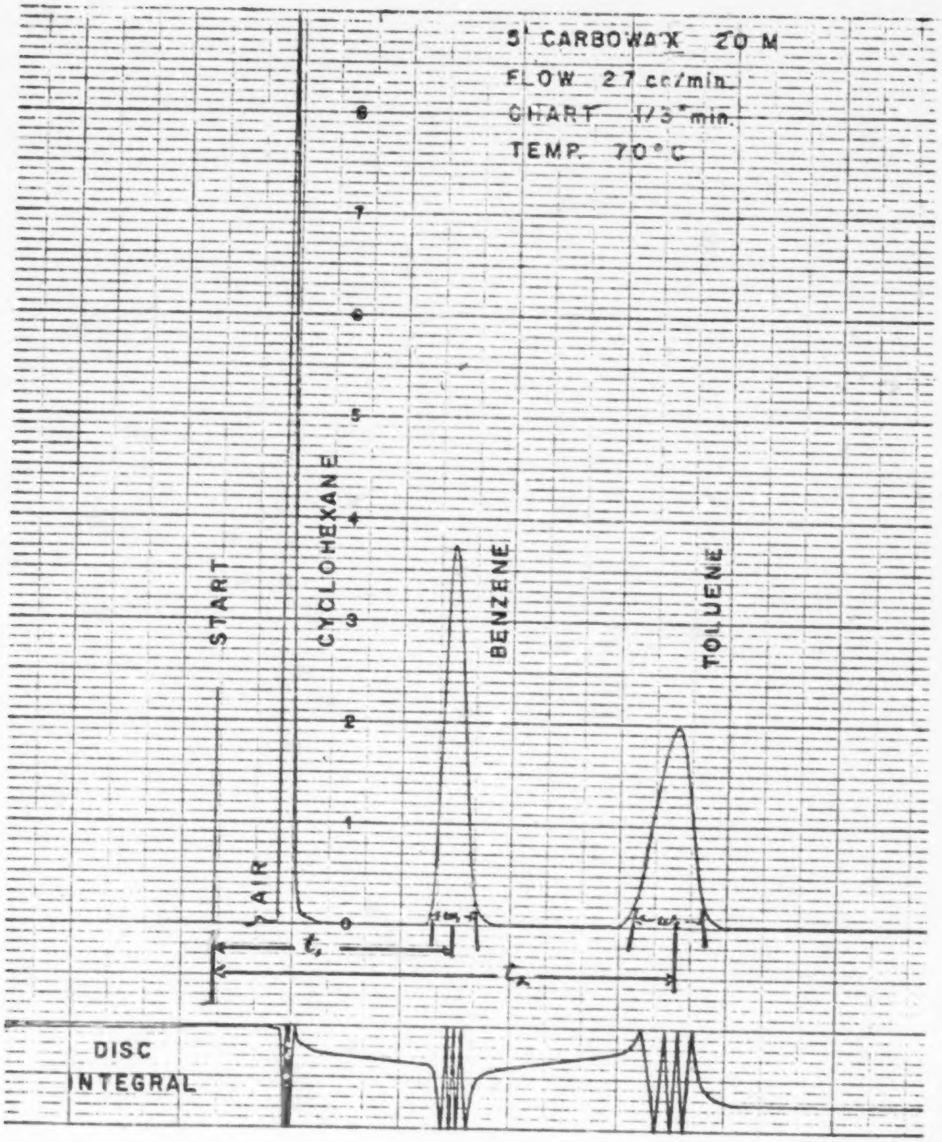

5' CARBOWAX 20 M
FLOW 27 cc/min.
CHART 1/3" min.
TEMP. 70° C
8
7
6
5
4
3
2
1
0
START
AIR
CYCLOHEXANE
BENZENE
TOLUENE
t_1
t_2
w_1
w_2
DISC
INTEGRAL

Figure 3.

GAS-LIQUID CHROMATOGRAPHY BIBLIOGRAPHY

Books

dal Nogare, S. and Juvet, R. Gas Liquid Chromatography. Interscience Publishers, New York, 1962. A general book, excellent section on thermal conductivity.

Littlewood, A.B. Gas Chromatography. Academic Press, New York, 1962. A general book with lots of applications and tables of data. Very readable.

Purnell, H. Gas Chromatography. John Wiley and Son, New York, 1962. A much more theoretical book than the other two, excellent sections on the physical chemistry of gas chromatography.

Kaiser, R. (translated by P.H. Scott). Gas Phase Chromatography. Butterworths, Washington, 1963. This work is in three volumes and is very concise and complete. Volume III has a great deal of retention data.

Volume I - Gas Chromatography
Volume II - Capillary Chromatography
Volume III - Tables for Gas Chromatography

Collected Papers from Symposia held by Gas Chromatography Discussion Groups. These are published under the title "Gas Chromatography 19__." The even years, which were held in Europe, are published by Butterworths, Washington, D.C.; the odd years, which were sponsored in this country by ISA, are published by Academic Press, New York. The editions are edited by:

1957 - Coates, V.J. et al.
1958 - Desty, D.H.
1959 - Noebels, H.J. et al.
1960 - Scott, R.P.W.
1961 - Brenner, N. et al.
1962 - van Swaay, M.
1963 - Fowler, L.

Though these books are a series of papers, many of the basic advances in the state of the art were reported at these meetings. The results of discussions which followed the papers are also included.

Journals

A good share of all papers dealing with gas chromatography are published in three journals, Analytical Chemistry, Journal of Chromatography, and Journal of Gas Chromatography. Some of the earlier articles appeared in Nature.

Abstracts

Gas chromatographers have been fortunate to have their own sets of abstracts. These are available from two sources, in card form from Preston Abstract Service, Evanston, Illinois, and in bound form as "Gas Chromatography Abstracts" edited by Knapman, C.E.H., et al., The Institute of Petroleum, London, 1958, -59, -60, -61, -62, -63.

Manufacturer's Literature

This is a very good source of information, especially about new developments. "Facts and Methods", F. and M. Scientific Company, Avondale, Pennsylvania. "Research Notes" and "Reviews and Previews", Wilkens Instrument Company, Walnut Creek, California.

"Chromatogram", Barber Colman Company, Rockford, Illinois.

"Instrument News", the Perkin-Elmer Company, Norwalk, Connecticut.

This outline was prepared by J.W.Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

THEORY OF GAS CHROMATOGRAPHY PEAK BROADENING

(PART III)

I THEORY

The equation which expresses the contribution of column performance to plate height was first proposed by van Deemter and bears his name. In the simple form it is:

$HETP = A + \frac{B}{u} + Cu$ where u is the linear gas velocity and A, B, and C are constants for a given column. A plot of HETP vs. u, called a van Deemter plot, (Figure 1) is frequently used to establish proper flow rates.

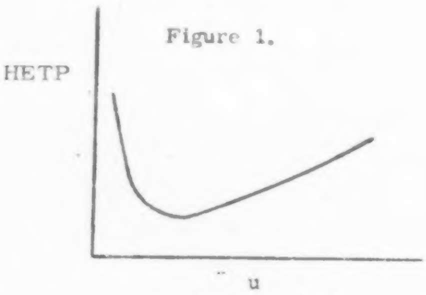


Figure 1.

van Deemter plot

This expression, though not precisely correct, is useful because it gives a concise description of efficiency as a function of flow rate and can be used to eliminate flow as a variable and discuss other column functions in terms of A, B, and C. The A term, which is also given as $A = 2\lambda d_p$, or a constant times the particle diameter, is the limiting case when u is zero. The constant λ describes the dispersion of sample crossing a particle diameter and has a value of about 2 for common columns. The B term, often called the gas term, describes the diffusion of the solute in the gas phase and is a function of the vicsocity of the gas and the diffusivity of the solute. C is a term which describes the slowness of mass transfer between the liquid and gas phases, and is an acknowledgment that this equilibrium is not instantaneous. Purnell, Golay, Giddings and others have pointed out more exact expressions and modifications of the van Deemter equation. These are especially needed in capillary and highly efficient packed columns. However, the simple form is quite adequate for understanding the factors involved and describes normal packed columns very well.

II SOURCES OF PEAK BROADENING

There are many sources contributing to peak broadening in a chromatographic system. These can be divided into two classes; those due to faults in the instrument and those due to the column.

A Instrumental Broadening

1. Injector - The purpose of the injector is to vaporize the sample and introduce it into the carrier gas as a slug. Poor injection may be due to insufficient heat to vaporize the sample, excessive volume in the injector, or poor sweeping of the chamber by the carrier gas.

2. Fittings - Connections and fittings frequently have high dead volumes and abrupt changes in size which cause excessive mixing and peak broadening.

3. Detector - Detection systems with high volumes can cause peak broadening.

4. Non-uniform temperature in the column - ovens, especially long ones, often have large temperature gradients in them. As a result the various parts of the column are at different temperatures, hence operating under different conditions.

B Column Parameters

1 Natural band width - In the development of the plate concept we arrived at a Gaussian shaped curve. This is the minimum width for an isothermal chromatogram in the ideal case.

2 Longitudinal diffusion - This is the "B" term in the van Deemter equation and takes place almost entirely in the gas phase. The peak broadens, remains symmetrical.

3 Non-instantaneous vapor equilibrium - This is the van Deemter "C" term and broadens peaks because of the finite time needed for mass transfer from the gas to the liquid phase. This results in sharp fronts and tailing backs.

4 Packing geometry - The packing in the column is frequently not uniform and thus we have non-uniform gas flow. The smaller particles tend to go to the column center, the large ones to the wall. Also "race tract" effects due to tightly coiled large columns frequently result in tailing fronts, sharp backs.

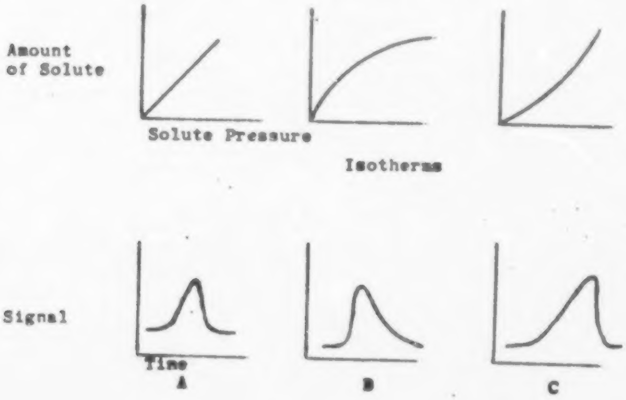
5 Non-linear isotherms - The ideal case assumes that a plot of solute amount vs. solute partial pressure is linear. Deviations may be either concave or convex. (Figure 2.)

6 Sample overload - When sample size exceeds the capacity of the column one gets into a non-linear portion of the isotherm. Condition 5-B is the most common with a sharp leading edge and a long tail.

7 Operating at a non-optimum linear velocity - This condition exists when operating outside the broad minimum of the van Deemter plot. When a column was a high pressure drip, only a portion of the column is operating at the optimum linear velocity.

8 Amount of liquid phase - Highly loaded columns are less efficient due to the higher value of the "C" term. However, low loading reduces capacity and hence sample size (6).

Figure 2.



Corresponding Chromatograms

9 Particle size and shape - The van Deemter A term. Particles should be uniform in size and shape; smaller sizes give more efficient columns until pressure drop becomes a limiting factor.

10 Column diameter - Efficiency decreases as column diameter increases because of non-uniform flow and temperature distribution. Special techniques such as mixing sections will help to overcome this.

11 Column length - The number of plates increases linearly with length until pressure drop becomes excessive.

12 Carrier gas - The optimum linear velocity changes with the carrier gas. However, this may be adjusted, and the choice of carrier is usually based on other criteria.

It can be seen that the final column design is always a series of compromises. The important factor is to have a system which will do the required analysis in the shortest possible time. Scott pictures the three related parameters, speed, resolution, and sample size, as being a triangle. (Figure 3.) Fixing any two will determine the third.

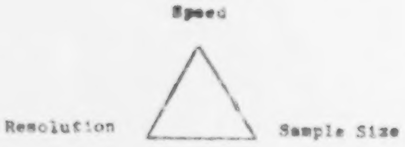


Figure 3.

This outline was prepared by J.W. Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

CHARACTERISTICS OF ELECTRON CAPTURE AND OTHER IONIZATION DETECTION SYSTEMS

Any gas chromatographic detection system consists of a sensing element and various electrical amplifiers, attenuators, recorders and integrators used to measure and record the signal from this sensor. Some detection systems, such as the flame ionization detector, respond to some common molecular parameter and the response is known for many compounds. Other detectors, such as the electron capture detector, are very specific in their response to electronegative compounds and have virtually no response for other substances.

One family of detectors is based on measuring the change in electrical properties of gas conducting electricity. These are based on the principle that if a gas is made to conduct electricity, then the system is very sensitive to small changes in the composition of the gas. Such detector systems include:

A Argon ionization system which works on the basis of using a radioactive source to raise the argon carrier to a metastable state, then observe the ion current generated by the ionization of other molecules as the argon metastables decay.

B Cross section ionization which works on the basis of radioactive bombardment of the gas in a detector and measuring the ion current. Hydrogen is often used as a carrier, since it has the smallest cross section and hence is ionized to the least extent. Materials with higher cross sections will have much more chance of being ionized, and hence produce positive currents.

C "Helium" detector which is used for determining very low levels of materials in a helium matrix.

D Electron Capture Detector

1 Radioactive source, which ionizes nitrogen carrier gas, producing thermal ions and large current. When electronegative sample enters the cell, it "captures" some of these electrons before they reach the anode resulting in a decrease in ion current.

2 Photoionization source which produces thermal electrons which are captured as above.

E Flame emission detectors, in which the spectral energy of a substance, such as phosphorus or sulfur, is examined with a photomultiplier.

F Thermionic emission detectors in which an alkali metal, in or near the flame, changes its rate of volatization and hence the number of ions present when halogens or phosphorus are introduced into the flame.

This outline was prepared by J.W. Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

THE ELECTRON CAPTURE DETECTOR

I INTRODUCTION AND HISTORICAL BACKGROUND

II DESIGN AND CONSTRUCTION

A Parallel Plate

B Concentric Tube

C Quasi-Concentric Tube

III RADIOACTIVE SOURCES (Commonly Used)

A Tritium

B Radium

C Ni-63

IV OPERATIONAL PARAMETERS

A D.C. Mode

B Pulse Mode

C Flow Rate

D Temperature

E Carrier Gas

F System Characteristics for Optimum Operation

G Column Temperature Programming

This outline was prepared by R.K. Stevens, Chief, Field Instrumentation Unit, Methods Development & Standardization Section, Chemical & Physical Research & Developement Program, NCAPC.

THE FLAME PHOTOMETRIC DETECTOR

I INTRODUCTION AND HISTORICAL BACKGROUND

II THEORY OF OPERATION

III PERFORMANCE

- A Sensitivity
- B Response Specificity
  - 1 Phosphorous response
  - 2 Sulfur response
- C Flame Ionization Response

IV APPLICATION OF THE FLAME PHOTOMETRIC DETECTOR TO PESTICIDE RESIDUE PROBLEMS

V APPLICATION OF THE FLAME PHOTOMETRIC DETECTOR TO CONTINUOUS OR G.C. INTERRUPTED ANALYSIS OF ATMOSPHERIC SULFUR GASES.

This outline was prepared by R.K. Stevens, Chief, Field Instrumentation Unit, Methods Development & Standardization Section, Chemical & Physical Research & Development Program, NCAPC.

CHARACTERISTICS OF THE FLAME IONIZATION DETECTOR

When gases burn in a flame and become sufficiently hot a small percentage of the molecules ionize and give rise to an electrical conductivity within the flame. When an organic molecule enters this flame it causes this conductivity to increase. This increased conductivity is measured and recorded. Thus, we have the basis for a very sensitive method for the detection of organic material. The credit for this observation is given to Harley, Nel, and Pretorius and to McWilliam and Dewar(1, 2, 3)

The exact mechanism within the flame which is responsible for the conductivity has been the subject of considerable smoke and very little light and for this reason will not be discussed here. Interested parties will want to consider references 4, 5, 6, 7, and 8 for further information on this point. It should be mentioned however that the efficiency of the flame ionization detector (FID) is very small having an upper limit of approximately 0.01% and ranging down to 0.0001% for most common commercial detectors.

The electrical characteristics of the FID should be mentioned since they give us a clue as to its best mode of operation. In normal practice one usually puts 200 to 300 volts across the electrodes to collect the ions. The resistance of a hydrogen flame is of the order of 10^{14} ohms while the background current is approximately 10^{-12} amperes and the signal current may run up to 10^{7} amperes. The measurement of such small currents demands that we use a high impedance amplifier (electrometer) and some care in the design of the FID itself along with the associated circuity.

SENSITIVITY AND LINEARITY

It should be kept in mind that the FID is a mass flow detector rather than a concentration detector. This means that the response of the flame is dependent on the number of grams of carbon which enter the flame per second rather than the concentration of carbon in the carrier gas entering the flame. The limit of detection for this device is expressed in such terms as 1 in 10^{9} molar, one part per billion, 2×10^{-13} gram atoms of carbon, or 5×10^{-12} grams of carbon per second. The linearity of the flame commences at detectability and runs to at least 0.5% while the dynamic range covers four to six orders of magnitude.

CALIBRATION

This device demands careful calibration and operation for good quantitative results as shown by the fact that the molar response for 2,2,4-trimethylpentane is 30% greater than that for ethyl benzene and 15% greater than that for n-octane (all C_8 molecules).

One usually calibrates a FID by determining response factors for the compounds of interest. This response depends on geometry and operating conditions of the FID and is usually expressed in coulombs per gram of carbon or in coulombs per gram atom of carbon. In the ultimate analysis what we wish to know is the absolute or relative amount of material leaving the chromatographic column. This amount is the integral of the detector current with respect to time providing the detector signal and mass flow rate are linear over the range in question.

Figure 1 and Equation 1 illustrate what we are measuring.

$$I = r \times g \times t^{-1} \qquad (1)$$

where:

I = amperes

r = coulombs/gram

g = grams of carbon

t = time in seconds

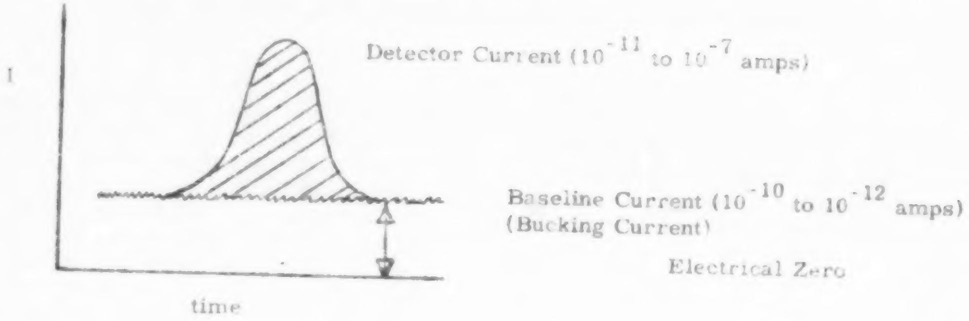


Figure 1

OPERATING PARAMETERS OF THE FID

In general the electrode geometry and polarizing voltage for the detector are determined by the manufacturer and you have very little control over them. This is, however, an important point and should not be overlooked in the purchase of new equipment.

GASES USED

When using a FID and working in the low parts per billion region certain problems become acute. Foremost among these is the need for "clean" gases (hydrogen, air, nitrogen or helium). Using proper techniques(6) it is possible to get sensitivity to the low parts per billion range.

For hydrogen: Use a palladium-silver diffusion purifier or a liquid nitrogen cold trap changed with Davison 912 silica gel.

For air: Air taken through a combustion furnace at 825° C and then over a drying agent such as Drierite.

For helium: Use a liquid nitrogen trap; still some residual methane gets through.

TYPICAL GAS FLOW RATES FOR FID

The values listed below are "average" values. For maximum sensitivity one must experimentally determine what the best flow rates are for each individual detector.

Scavenger gas: Either air or oxygen may be used at a flow rate of between 200 and 500 cc/minute depending on the geometry and flow patterns within the detector.

Fuel gas: Hydrogen is normally used at a rate given by:

$$H_2 = \frac{1}{3}(\text{carrier gas} + 10)\ \text{cc/minute}$$

Carrier gas: Usually helium or nitrogen is used at a rate given by:

$$\text{Carrier gas} = 3(H_2\,\text{flow} - 10)\ \text{cc/minute}$$

Many times this flow rate is determined by chromatographic conditions and it may be necessary to add "make up" gas between the end of the column and the flame detector.

OPTIMIZING FID PERFORMANCE

1) Dependence of ion current on polarizing voltage
2) Dependence of ion current on mass flow rate of sample
3) Dependence of ion current on scavenger gas rate
4) Dependence of ion current on fuel gas rate
5) Dependence of normalization factors on fuel gas rate

CONCLUSION

A great deal of information is available today on the design and operation of flame detectors. In order to do good quantitative work the operator of such a detector must take the time to understand its operation and limitations. Used with care this detector will give very good results - however, if used in an indiscriminate manner it will lead to results which may contain large errors. The type of work you do will depend on your understanding of this detector.(10)

REFERENCES

1 McWilliam, I.C. and Dewar, R.A. Nature. 181, 760 (1958).

2 Harley, J.W., Nel, W. and Pretorius, V. Nature. 181, 177 (1958).

3 McWilliam, I.G. and Dewar, R.A. Gas Chromatography. (Ed. D.H. Desty), Butterworth, London. p. 142 (1958).

4 Lewis, B. and von Elbe, G. Combustion Flames and Explosions. Academic Press, New York. p. 206 (1951).

5 Sternberg, J.C., Gallaway, W.S. and Jones, D.T.L. International Gas Chromatography Symp. E. Lansing, Mich. (1961).

6 Calcote, H.F. and King, I.R. Fifth International Symposium on Combustion. Reinhold, New York. p. 423 (1955).

7 Calcote, H.F. Combustion and Flame. 1, 385 (1957).

8 Calcote, H.F. Ion Production and Removal in Flames. Eighth International Symposium on Combustion. Pasadena, California, August 29 - September 2, 1960.

9 Prescott, D.O. and Wise, H.L. Journal of Gas Chromatography. 4, pp. 80 - 84 February 1966.

10 Bruderreck, H., Schneider, W. and Halasz, I. Analytical Chemistry. 36, pp. 461 - 473 March 1964.

This outline was prepared by W.E. Baitinger, Department of Chemistry, Purdue University, Lafayette, Indiana.

USE OF A MICROCOULOMETRIC TITRATING SYSTEM AS A GAS CHROMATOGRAPH ANALYZER

I PRINCIPLE BEHIND THE MICROCOULOMETRIC APPROACH TO RESIDUE ANALYSIS

A Specificity

B Sensitivity

C Stoichiometry

II THE MICROCOULOMETRIC TITRATING SYSTEM (MCTS)

A Pyrolysis Furnace

B Titration Cell

C Coulometer

D Recorder

III PYROLYSIS FURNACE DESIGN

A Inlet Oven

B GLC Inlet

  1 Requirements

  2 Coaxial flow systems

C Furnace(s)--S-100, S-200

IV TITRATION CELL DESIGN

A Central Titration Chamber - Gas Inlet

B Reference Electrode Side Arm

C Generator Electrode Side Arm

D Cell Cap - Sensor Electrode and Generator Electrode

V MICROCOULOMETER DESIGN

A Input Circuit

B Amplifier

C Output Circuit

D Measuring Circuit

E Calculation

VI OPERATIONAL MODES

A Mode I

B Mode II

This outline was prepared by James A. McNulty, Sales Manager, AI Division Dohrmann Instruments Company, Mountain View, California.

PREPARING GAS CHROMATOGRAPHY COLUMNS

I INTRODUCTION

A The object of this laboratory demonstration is to familiarize the student with columns and packing materials, and procedures used in preparation of columns for pesticide residue analyses using the electron affinity detector.

B Materials

1 Columns

a Stainless steel

b Aluminum

c Quartz

d Glass

2 Solid support

a Diatomaceous earth

1) Chromosorb, regular
2) Chromosorb P

a) Pink
b) Calcinated (Powdered by heating at high temperatures)

3) Chromosorb W

a) White
b) Flux-calcinated (Heated at high temperatures with about 3% sodium carbonate)

4) Anakrom

a) White
b) Flux-calcinated
c) Silanized

5) Acid washed chromosorbs

a) Washed with concentrated hydrochloric acid
b) Removes traces of aluminum, iron, magnesium, and calcium, which are present in diatomaceous earth

b Firebrick

1) Firebrick, regular
2) Firebrick, acid washed

c Polytetrafluoroethylene (Teflon)

1) Fluoropak
2) Teflon 6
3) Neutraport
4) Haloport

3 Stationary phase (Immobile phase)

a Silicone Dow 11

b Silicone SE-30

c Epikote

d Apiezon L

II PROCEDURES USED TO IMPROVE THE SOLID SUPPORT

A Separation of Materials into Narrow Range Particle Sizes

B Removal of Foreign Materials

1 Acid washing

2 Alkali washing

C Deactivation - Covering of Active Absorption Sites

1 High temperature

2 Sodium carbonate

3 Silicon dioxide

4 Hexamethyldisilazene coating

5 Epon coating

III COLUMN PREPARATION PROCEDURE

A Column

1 Select appropriate material.

2 Cut to desired length.

3 Weigh empty column and record weight.

4 Calculate the volume of the column in cubic centimeters (Quoted column diameter is outside diameter).

B Preparation of Packing Materials

1 Select the solid support.

2 Weigh out this material slightly in excess of that required to pack the column.

3 Select the desired stationary phase material.

4 Calculate the desired amount of stationary phase to use with the previously weighed solid support.

5 Dissolve the stationary phase material in an appropriate volatile solvent, such as ethyl acetate, in sufficient quantity to completely cover the solid support, leaving an excess of liquid phase.

6 Thoroughly mix the solid support and solvent containing the stationary phase.

7 Completely remove the solvent by means of a steam bath or rotating evaporator, with constant mixing.

8 Place the prepared packing material in a drying oven for several hours or overnight. A good general rule is to dry at about 90° C, temperature should not be allowed to reach 100° C.

C Packing the Column

1 Lightly pack one end of the column with a plug of glass wool.

2 By means of a tube, attach a small funnel to the opposite end of the column.

3 With a graduated cylinder, measure the volume of packing material which is equal to the volume of the column, tapping the cylinder to compact the material.

4 Weigh this volume of packing material and record the weight.

5 Slowly add this material to the column, running a small hand vibrator up and down the column or tapping the column on the floor to compact the packing.

6 Close the open end of the column with a plug of glass wool.

7 Attach the fittings to the column and install in the chromatograph column oven.

8 Check the carrier gas flow through the column at the operating gas pressure.

9 With the carrier gas flowing, condition the column by heating at a temperature about 50° C higher than operating temperature.

10 The period of time required for conditioning a column ranges from about 12 - 72 hours depending upon the packing materials used.

11 Appreciable up-scale drifting of the baseline is indicative of improper or insufficient conditioning of the column.

REFERENCES

1 Baitinger, W.E. and Amy, J.W., Stationary Phases Suitable for Gas Chromatography. Purdue University. 1962.

2 Amy, J.W., Procedure for Packing Columns. Purdue University. 1962.

3 Schafer, M.L. Private Communication.

4 Wilkins Instrument Co. Aerograph Previews and Reviews on Gas Chromatography. October 1961.

TROUBLE SHOOTING THE GAS CHROMATOGRAPH

I ROUTINE FOR DAILY OPERATION OF GAS CHROMATOGRAPH

A Turn on all electronics for warm-up.

B Check system for gas leaks and then adjust gas flow.

C Check recorder zero and note baseline at various sensitivities.

D Inject standards and compare with previous runs.

II PROBLEMS AND CAUSES (ELECTRON CAPTURE)

A Erratic baseline noise. (In excess of 4% of half of maximum sensitivity).

1 Column operating temperature too high.

2 Column substrate coating weight excessive (in excess of 7%).

3 Columns insufficiently conditioned.

4 Contaminated system.

5 Insufficient bakeout time with pure gas flowing.

6 Internal recorder signal connections improper.

7 Signal cable loose.

8 Loose ground.

9 Effluent end of Electron Capture Detector exposed to turbulent air.

10 Intermittent Electron Capture Detector heater.

11 Cracked Electron Capture Detector housing.

12 Effluent end of detector shorting to case.

B Slow Regular Noise

1 Insufficient pressure differential between carrier supply regulator and column head pressure at the maximum analytical column operating temperature.

2 Column temperature too high.

3 Condensation somewhere between end of column and effluent end of Electron Capture Detector.

4 Defective carrier flow regulator.

C Insufficient Sensitivity of Response

1 Using metal columns

2 Septum leaking

3 Bad leak

4 Cold spot

5 Diluent flow too high or too low.

6 Improper carrier gas.

7 Carrier gas contaminated with water.

8 Carrier gas contaminated with oxygen.

9 Operating Electron Capture Detector electrode potential in DC Mode incorrectly set.

10 Recorder incorrectly connected to instrument signal output terminals.

11 Depleted (weak) source element.

12 Pulse rate too fast, in pulse mode operation.

13 Pulse generator not operating, in pulse mode operation.

14 Cracked Electron Capture Detector housing.

15 Leaking Electron Capture Detector housing.

D Baseline Drift Upscale

1 Column temperature programming (thermal upscan).

E Baseline Drift Downscale

1 Contaminated sample system.

a The "proper solvent" is benzene, hexane, or similar non-adsorbing hydrocarbon solvent.

b Chlorinated or other electron absorbing solvents should be avoided except as a last resort in cases of extreme contamination.

c More generally the following solvents and cleaning method must be avoided:

1) Ethyl or Methyl Alcohol

2) Water

3) Organic Acids, Acetic Acid, etc.

4) Phenolic Solutions

5) Anything containing Labile, Acting Hydrogen.

6) Scrubbing the source by ultrasonic cleaning.

2 Column bleed.

F Excessive Tailing From Sample Solvent Peak

1 Premature vaporization of sample at in-side end of septum.

2 Solvent

3 Diluent gas flow rate insufficient.

4 Sample quantity excessive.

III PROBLEMS AND CAUSES (MICROCOULOMETRIC)

A Positive High Baseline

1 Titratable material entering cell.

B Negative Baseline

1 Silver from silver acetate electrode entering cell.

C Noisy Baseline

1 Stirring bar

D Baseline goes negative at irregular intervals and is slow returning

1 Excess silver in cell

E Erratic baseline

1 Temperature of cell varying.

F Peaks Overshoot

1 Under damping.

G Peaks overshoot but system acts over damped.

1 Dirty cell

H Peaks tail

1 Over damped

I Odd Peaks

1 Contaminated system

J Peaks Break-up

1 Contaminated system

K Low Recoveries

1 Leaking system

2 Improper combustion

This outline was prepared by J.I. Teasley, Supervisory Chemist, Southeast Water Laboratory, FWQA, Athens, Georgia.

QUANTITATION IN GAS CHROMATOGRAPHY

I INTRODUCTION

One of the factors which has led to gas chromatography's rapid development in the field of instrument analysis is the quantitative precision with which samples can be analyzed. There are several equally important factors involved in quantitative analysis. These are: Accurate sample introduction, constant operating parameters, accuracy of peak area measurement, sensitivity factors of individual compounds, linearity of detector and columns which give well resolved peaks.

II CALIBRATION PROCEDURE

A Peak Height and Peak Area

Quantitative work is based on peak height or area. Peak height measurement is more rapid than peak area; however, plots of peak height vs. sample size are more non-linear than corresponding plots for peak area. This is because peak heights and widths are frequently dependent on sample size and sample feed volume, however; total area is not. Peak heights are generally used if samples are less than 10 µg for packed columns and 0.1 µg for capillary columns.

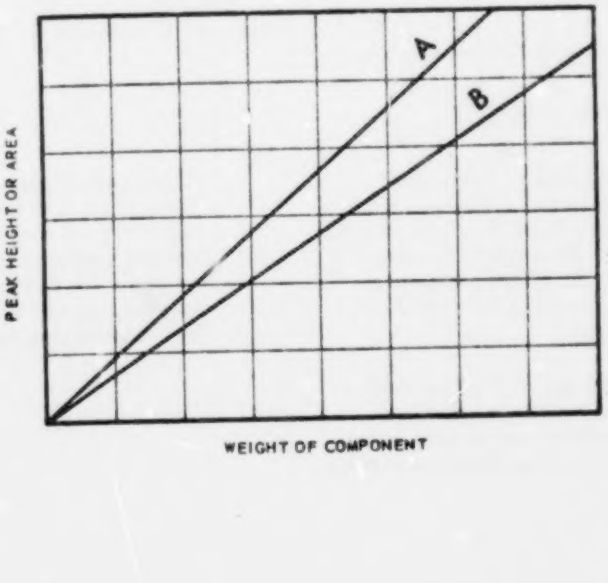
B Direct Calibration

Blends of components in question are prepared and chromatographed. The value of peak heights or areas are then plotted against the weight of sample injected. The unknown sample is then injected and its peak height or area is compared to that of the standard.

The main disadvantages of direct calibration are that the precise amount of sample injected must be known and that calibration is time consuming. Also, the sensitivity of the detector must remain constant from run to run and day to day in order to compare results with the calibration graph.

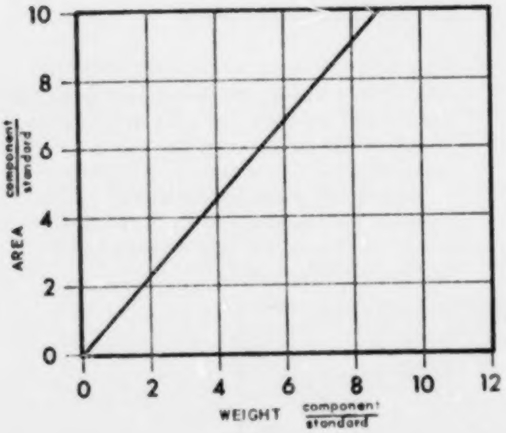
C Internal Standardization

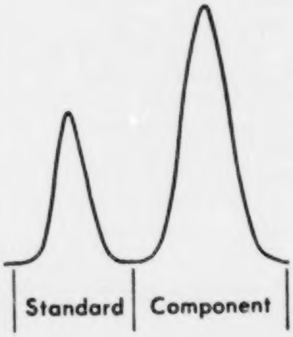
This is a common procedure used in biomedical analyses, such as alcohol in blood, and steroids in serum and urine. Known weight ratios of the component in question and a standard (marker) are prepared and chromatographed. The component/standard area ratios obtained are then plotted vs. the component/standard weight ratios.



To the unknown, add a known volume concentration of the standard. This mixture is then chromatographed and the component/standard area ratios are determined. By determining the corresponding component/standard weight ratio from the graph, the unknown amount of the component can be determined.

e. g.: To the unknown, add 5 ml of a solution containing the standard whose concentration is 100 µg/ml. Upon chromatographing the mixture, the area ratio was found to be 8; therefore, the weight ratio is 7 (see graph). Knowing standard concentration to be 100 µg/ml, then the component concentration is 7 × 100 µg/ml. Since we added 5 ml of standard solution, then the total amount of the unknown is 5 ml × 700 $\frac{\mu g}{ml}$ = 3500 µg or 3.5 mg.



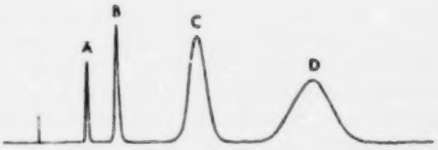


A particular advantage of using the internal standardization method is that the amount injected need not be accurately measured. Also, the detector response need not be known or remain constant since any change in sensitivity will not affect the area ratio.

The chief disadvantage of this method is the difficulty in finding a standard that does not interfere with a component in the sample. It is also time consuming.

D Internal Normalization

This is used when only approximate data are required. Assuming all the components have been eluted, the percent composition of a component within a mixture is its area divided by the sum of the areas.



% Composition A = $100 \left(\frac{A}{A + B + C + D} \right)$

This method also assumes that area percent equals weight percent. This may only be true when analyzing close boiling components of a homologous series. To obtain the weight percent, it is necessary to multiply each area by a correction factor.

III NORMALIZATION OF AREAS (CORRECTION FACTORS)

A Normalization for Thermal Conductivity Detectors

This procedure relies on the fact that each compound has a unique thermal conductivity cell response. For quantitative analysis, the thermal conductivity of the sample should be approximately a linear function of its composition in the range concerned. From the areas under each peak and the relative responses that are characteristic of each peak, it is possible to determine the quantity of each component in the sample.

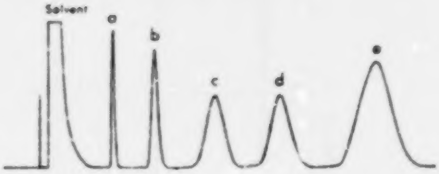
Sample calculation:

COMPOUND	AREA	RELATIVE T.C. RESPONSE PER MOLE (2)
Ethanol	5.0	72
Heptane	9.0	143
Benzene	4.0	100
Ethyl Acetate	7.0	111

			NORMALIZE		MOLE %
Ethanol	$\frac{5.0}{72}$	= 0.070	$\frac{0.070}{0.236}$	=	29.6
Heptane	$\frac{9.0}{143}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
Benzene	$\frac{4.0}{100}$	= 0.040	$\frac{0.040}{0.236}$	=	17.0
Ethyl Acetate	$\frac{7.0}{111}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
TOTAL		0.236			100.0%

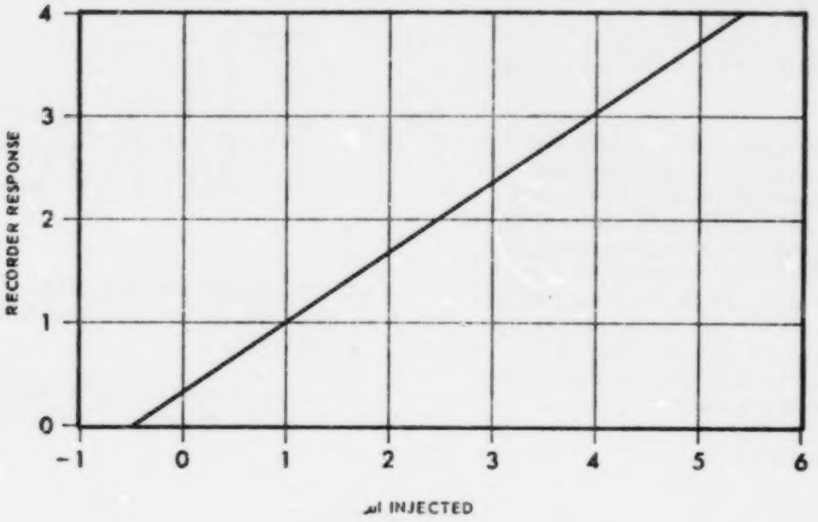
B Normalization for Mass Detectors

1 A standard solution of compounds a, b, c, d, and e in a solvent is prepared.



2 The weight percent is calculated and the known densities are used to convert percent.

3 Inject several samples and plot peak area (or height) vs. volume injected.



4 Note that intercept on "X" axis corresponds to an injection error of 0.5 µl. Therefore, actual amount injected = uncorrected + 0.5 µl. For 2.5 µl (corr.) injected, the density values are used to determine the actual weight W. injected.

5 The corresponding area of each peak is determined and the response in $cm^2/\mu g$ is determined.

6 Using compound "a" as standard (correction factor = 1), the corresponding correction factors are determined. See the Table on Page 8-6.

IV STATISTICAL TREATMENT OF DATA

A Accuracy and Precision Data (Reproducibility)(3, 4)

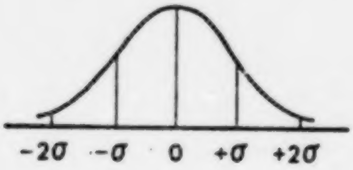
Accuracy is a measurement of the difference between the true value and the determined values. In those cases in which the true value is not known, it is necessary to express the exactness of a measurement in another way. This may be done by obtaining the average of a number of measurements and finding the difference (deviation) of each value from the average value. The magnitude of deviation is a measure of the precision of measurement.

It is seen that accuracy expresses the correctness of a measurement, whereas precision expresses the reproducibility of a measurement.

The accuracy and reproducibility achieved in gas chromatography depend on many things; among them, the correct choice of column, temperature, flow rate, sample size, detector, and injection system. Highest performance demands considerable understanding of the chromatographic process and of the effects of change in a large number of variables. However, even with ill-designed apparatus, analyses can usually be conducted with an accuracy better than ± 10 percent per component. When precautions are taken, an accuracy and reproducibility of about ± 1 percent per component is easily attainable.

B Errors

Errors fall into two classes. Determinate errors are those whose cause and magnitude can be determined. (Errors in method, apparatus, operator, etc.) Indeterminate errors are random errors which cannot be eliminated. The distribution of indeterminate errors follows the normal probability law as shown in the error curve. This curve shows that positive and negative deviations are equally probable and that small deviations occur much more frequently than large ones.



σ = Standard Deviation
$\pm\sigma$ = 68%
$\pm 2\sigma$ = 95%

V MEASUREMENT OF AREAS

A Methods

1 Cutting and weighing is a fairly accurate but tedious procedure. Besides the errors that may arise from thickness and moisture content of the paper, the chromatogram is destroyed. It is not satisfactory for estimating areas of overlapping peaks.

2 The planimeter is a devise whose acquisition is rarely justified. In addition to being very fatiguing, it is necessary to acquire considerable practice in its manipulation. The sensitivity of a normal planimeter is usually 10 mm^2, which is in many cases insufficient for analytical purposes.(5)

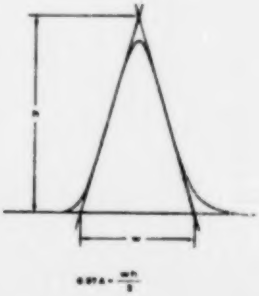
3 Integrators

a Mechanical integrators are exemplified by the Disc Integrator. This is a device which is attached to most strip chart recorders. The integrator pen tracing is displayed at the bottom of the chromatogram and the pen speed is proportional to the displacement of the recorder pen from baseline. (See triplicate runs of the nonanedecane mixture.) This is a frequently used, accurate device.

b Electronic integrators are devices which automatically print cumulative integrals of peak area. In addition to offering outstanding precision, electronic integrators automate chromatographic operations. This eliminates the time consuming necessity of the chemist keeping a watchful eye on the operation. Its biggest disadvantage is its cost.

4 Triangulation

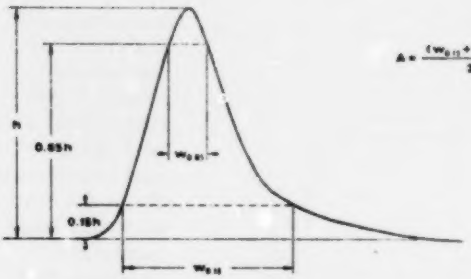
a Gaussian peaks



SAMPLE	WT. %	DENSITY	VOL. %	VOL. INJECTED, µl		W WT. INJECTED	A AREA	cm^2/ug	F CORRECTION FACTOR
		gm/cm^2		uncorr.	corr. (actual)	µg	cm^2		
Solvent	99.79	.88	99.781	1.99562	2.49453	2195.19	--	--	--
a*	.02	0.50	.035	.00070	.00087	.435	4.0	9.19	1.00
b	.03	0.75	.035	.00070	.00087	.653	6.5	9.95	1.08
c	.04	0.90	.0387	.00077	.00096	.864	7.6	8.79	.96
d	.04	0.90	.0387	.00077	.00096	.864	8.1	9.38	1.02
e	.08	1.00	.0704	.00141	.00176	1.760	15.0	8.52	.93
Total	100.00		100.00	2.00	2.50	2199.766			

*Correction factors calculated relative to "a" as standard.

b Tailing or leading peaks, trapezoidal construction.

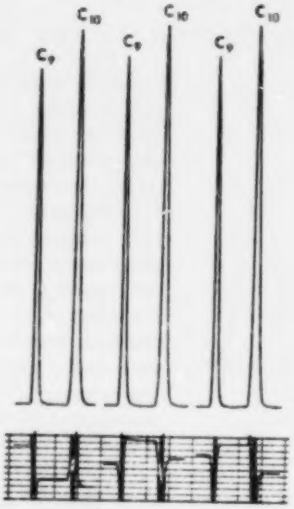


$$A = \frac{(w_{0.15} + w_{0.85})h}{2}$$

B Comparison of Integration Methods

Using several of the above methods on the triplicate runs of the nonanedecane mixture, the following table was obtained:

Triplicate analysis of nonane-decane mixture



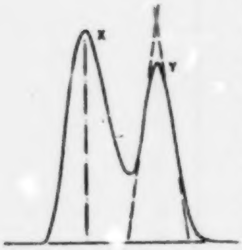
COMPARISON OF INTEGRATION METHODS

	#1	#2	#3	Avg.	σabs	σrel
MODEL 471 DIGITAL INTEGRATOR						
n-Nonane	39.156	39.150	39.193	39.166	0.0716	0.184%
n-Decane	60.844	60.850	60.807	60.834	0.0227	0.037%
DISC INTEGRATOR						
n-Nonane	39.33	38.97	38.91	39.07	0.22	0.56%
n-Decane	60.67	61.03	61.09	60.93	0.23	0.38%
TRIANGULATION						
n-Nonane	40.77	40.68	40.07	40.51	0.38	0.94%
n-Decane	59.23	59.32	59.93	59.49	0.39	0.66%
WEIGHING PAPER						
n-Nonane	42.58	41.73	42.83	42.38	0.58	1.37%
n-Decane	57.42	58.27	57.17	57.62	0.58	1.01%

Summary: Electronic digital integration is the most precise quantitation method; weighing paper is the least precise. Based upon this and other data in this publication, the electronic integrator give 2 to 5 times more precision than Disc Integration, 4 to 10 times more precision than triangulation, and 7 to 25 times more precision than cutting and weighing paper.

C Measurement of Overlapping Peaks

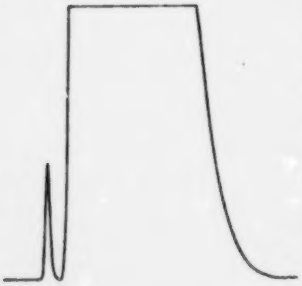
1 Components present in equal amounts.



Since broken line tangent to peak y intersects baseline after peak x has reached its maximum, then peak heights may be used. If broken line tangent to peak y intersects baseline before peak x has reached its maximum, then various known mixtures of x and y are prepared, chromatographed, and compared to the unknown x and y.

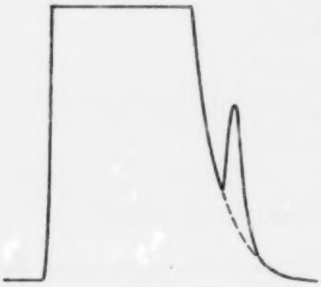
2 Trace analysis (less than 20 ppm)

a Trace component eluting before major peak.



In any trace analysis, one should choose a column and conditions which will allow the minor peak to elute before the major peak. This is easier to quantitate, and generally, peak height methods are used.

b Trace component on tail of major peak.



Assuming one has tried all conceivable columns and conditions, extend baseline as a continuation of the solvent peak and determine area. Compare this area with a synthetic mixture. Also, one can "spike" the sample with known amount of the trace component and compare area before and after.

BIBLIOGRAPHY

1 Aerograph Previews and Reviews August 1964 p. 6 .

2 Messner, A. E., Rosie, D. M., and Argabright, P. A. Analytical Chemistry 31, 230 1959 .

3 Aerograph Research Notes, Fall Issue, 1965.

4 Purnell, Howard Gas Chromatography, Wiley. 1962.

5 Lewin, S. Z. Journal of Chemical Education, 41, No. 4, A235-A259, April 1964.

This outline was prepared by E. J. Bonelli, Applications Manager, Varian Aerograph Company, Walnut Creek, California.

III OPTICAL METHODS (RADIATION) OF ANALYSIS

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24A

RECENT DEVELOPMENTS IN THE IDENTIFICATION OF OILS BY INSTRUMENTAL ANALYSES

Part 1

I INTRODUCTION

- A Health Hazards of Oil
- B Analytical Procedures
- C Need

II EXPERIMENTAL

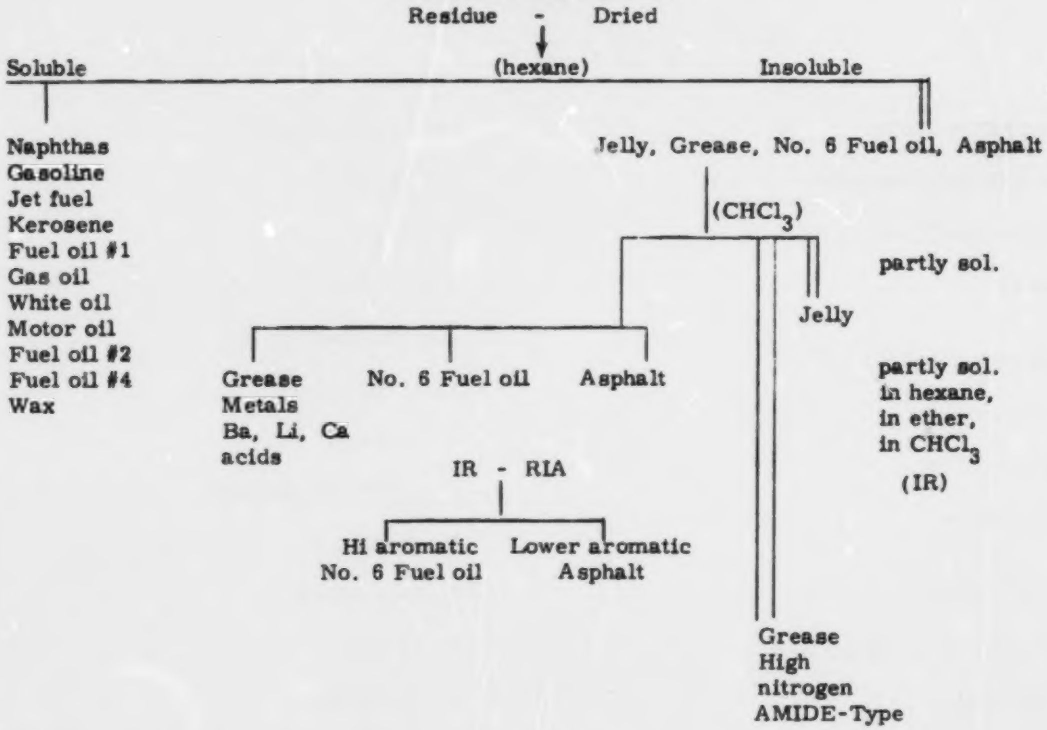
- A Sampling
- B Preparation
- C Variations

III ANALYSES

- A Characterization scheme
- B Solubility
- C Density
- D Initial B.P., final B.P.
- E Infrared Analyses
  - 1 Spectra
  - 2 Ratios
  - 3 Mixtures
- F Gas Chromatography
- G Case Histories
  - 1 Asphalts
  - 2 Heavy residual oils
  - 3 Heater oil
  - 4 Lubricating oil
  - 5 Xylenes
  - 6 Gasolines

This outline was prepared by F.K. Kawahara, Organic Chemist, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

Characterization Scheme


Residue - Dried
(hexane)
Soluble
Insoluble
Naphthas
Gasoline
Jet fuel
Kerosene
Fuel oil #1
Gas oil
White oil
Motor oil
Fuel oil #2
Fuel oil #4
Wax
Jelly, Grease, No. 6 Fuel oil, Asphalt
($CHCl_3$)
partly sol.
Jelly
partly sol. in hexane, in ether, in $CHCl_3$ (IR)
Grease
Metals
Ba, Li, Ca
acids
No. 6 Fuel oil
Asphalt
IR - RIA
Hi aromatic
No. 6 Fuel oil
Lower aromatic
Asphalt
Grease
High
nitrogen
AMIDE-Type

Residue - Dried

↓

Soluble (hexane)

↓

	I.B.P. - e.P.	API - gravity	Comment
High gravity naphtha	95° - 206°F	45 - 75	
Gasoline	96° - 408°F	58 - 62	
Low gravity naphtha	160° - 410°F	30 - 52	
Jet fuel	100° - 500°F	40 - 55	
Kerosene	355° - 575°F	40 - 46	
Fuel oil #1	360° - 625°F	>35	k. vis.
Gas oil	400° - 800°F	30 - 33	
White oil	-	29 - 32	I.R. paraffin
Motor oil	-	24 - 30	Zn, P, S
Fuel oil #2	370° - 625°F (90%)	>26	k. vis.
Fuel oil #4	420° - 683°F	9 - 36	k. vis.
Wax	MP = 145° - 190°F	34 - 39	Solid - IR paraffinic

ANALYSES OF OIL SLICKS

Part 2

I INTRODUCTION

A Foreword

Oil pollution has become a major problem in the coastal and surface waters of the United States and other countries. Discharges and spills from offshore industrial plants and commercial ships impart unpleasant taste and odor to potable water and to game fish and produce unsightly conditions which ruin beaches and recreational facilities. As predicted, carcinogens present in heavy oils, such as lube oils, heavy residual oils and asphalts, have already been found in flora and probably will be found in fish, etc.

The urgency of these problems requires that procedures for detection, characterization, and identification of the oil pollutants be developed to forewarn of the impending health hazards and to establish responsibility for violations of water quality standards.

In some cases, comparative examination of the infrared spectra of the oil slick collected from surface water with those of the suspect oil may be sufficient. In many cases eye witnesses are not available. Chemical analyses must be made to facilitate identifying the source of pollution. Thus a definite knowledge of the composition and character of the oil slick is vital as characterization is the preliminary step toward identification of the source of the pollutant of unknown origin. This procedure will aid in establishing the type of oil product and also in the identification of the source of the oil pollutant.

B Procedure

The complete procedure involves collection, extraction, drying, concentration and analyses.

C Equipment and Materials

Although conditions of heavier petroleum samples may vary, all are extracted with analytical grade dichloromethane (B.P. 40° C).

Dried samples of petroleum products and pollutants may be analyzed with the Perkin-Elmer 137 Infracord Spectrophotometer* that may be operated at slow-scan speed with a slit opening of 25 mm.

Salt prisms are polished on felt with the aid of rouge (Barnesite) mixed with ethanol.

For solvent removal, a commercially-available rotating vacuum flask evaporator or an all glass vacuum distillation system consisting of flask, Claisen head, condenser and receiver is suitable. Either of the two is attached to a dry ice trap leading to a mechanical vacuum pump or a building vacuum line.

II PROCEDURE

A Collection

Collect the oily material from the surface of the water with the aid of a large household mop with a ringer attachment. Before use, rinse the sponge thoroughly with dichloromethane. Pass the mop through the oil pool, squeeze and transfer by means of a widemouth funnel the absorbed oil into the collecting jar. If a mop is not available, sample the oil slick with aid of a widemouth bottle and transfer the contents to a larger bottle. Where possible, obtain reference samples of oil product from vessels and/or shore facilities.

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B Preservation of Sample

Protect the samples containing the water and oil against autoxidation and other chemical conversions by removal of air and exclusion of light. Add a tiny piece of dry ice to the sample. When the effervescing has stopped, seal the jar with a teflon lined cap. Preserve the samples in the refrigerator whenever possible. When nitrogen or an inert gas is not available, carefully fill with water the sample bottle to the top in order to displace much of the air.

C Sample Preparation for Analyses

To prepare a petroleum sample for analysis, extract the water sample with purified dichloromethane. Add 175 ml of solvent to a 500 ml separatory funnel. Add a portion amounting to about one-half to five grams of petroleum pollutant together with the large volume of sample water. Tilt the separatory funnel back and forth for one minute to extract the product. Set the funnel and contents on a ring stand placed under a well-ventilated hood. Draw the lower extract off and collect in a suitable Erlenmeyer flask. Repeat the operation, if necessary.

Place the combined extracts in the separatory funnel. Add 5 ml of distilled water and wash the extract by shaking gently. Allow the extract phase to separate from the water phase. Permit the lower solvent layer to pass dropwise through 5 grams of anhydrous sodium sulfate held in a No. 12 folded filter paper seated on a filtering funnel with a 75 mm diameter and 75 mm stem. Collect the filtered, dried pollutant extract in a 300 ml round bottom flask equipped with a standard tapered 24/40 ground glass joint.

NOTE: If the petroleum pollutant is considered to be more volatile than kerosene, centrifuge the sample if sufficient petroleum material is obtainable. Use a special syringe for the removal of the top phase. Transfer the contents from the needle syringe to a micro, conical, graduated, centrifuge tube of 2.5 ml capacity. Add a few grains of anhydrous sodium sulfate to dry the transferred light petroleum pollutant.

D Concentration

Attach to a rotating-type, vacuum evaporator, the round bottom flask with dried dichloromethane extract of the petroleum pollutant. If the amount of residue is small, rinse thoroughly the sides of the flask with about 5 to 10 ml of dichloromethane. Transfer the contents to a 20 ml conical, graduated centrifuge tube. Evaporate off the transferring solvent. If upon infrared analyses bands appear at 7.85μ and 13.55μ, continue drying until they no longer appear.

E Solubility Studies

From the thoroughly dried residue, free of water and extracting solvent, take about 0.2 gram portion and place into a 20 ml vial or test tube. Add seven (7) ml of hexane and stir the mixture with a glass rod. Observe whether the transferred residue is entirely soluble in hexane. Repeat the test to determine the solubility in seven (7) ml of diethyl ether. Determine the solubility in chloroform.

If the material is soluble in each of the three solvents, assume that the unidentified material is one of the following: light or heavy naphtha, kerosene, gas oil, white oil, cutting oil, motor oil, diesel oil or jet fuel. If the material is insoluble in hexane and in ether, but readily soluble in chloroform, assume that it is a grease, a heavy residual oil (No. 6, No. 5 or No. 4) or an asphalt. Refer to Table 1 on Solubility of Petroleum Products.

TABLE 1

SOLUBILITY OF PETROLEUM PRODUCTS

Product	Hexane	Ether	Chloroform
Light naphtha	VS	VS	VS
Heavy naphtha	VS	VS	VS
Gasoline	VS	VS	VS
Jet fuel	VS	VS	VS
Kerosene	VS	VS	VS
Gas oil	VS	VS	VS
Diesel oil	VS	VS	VS
White oil	VS	VS	VS
Cutting oil	S	S	S
Motor oil	S	S	S
Paraffin wax	S	S	S
White petroleum jelly	PS	PS	PS
Grease	I	I	S
Residual fuel oil	I	I	S
Asphalt feed stock	I	I	S

F Determination of API Gravity

Measure the density of the oil by the pycnometric methods, ASTM D-941 or ASTM D-1217. Weigh the dry, empty pycnometer (We). Weigh the same pycnometer when it is filled with oil (Wo). Empty and dry the pycnometer. Weigh the pycnometer when it is filled with water (Ww). Calculate the density, using the following formula:

$$\text{Density} = \frac{Wo - We}{Ww - We}$$

Compare the density with those given for petroleum products listed in Table 2.

III CHARACTERIZATION

A Chromatographic Analyses

For the identity of classified, volatile petroleum products, such as naphtha, gasoline, jet fuel and kerosene, analyze by means of flame ionization detection gas chromatograph the dried recovered residual material.

B Infrared Analyses

As greases, heavy residual fuel oil, and asphalt are less volatile, compare with the reference spectrum the unidentified spectrum obtained by infrared spectrophotometry. (If the unknown has been exposed to air and sunlight, consider possible changes due to peroxidation. Carbonyls will appear at about 5.75 μ to 5.85 μ and ethers at about 8.7 to 9.4 μ.) For viscous oils and asphalt, place the material between two salt plates. Place the plates on the crystal holder which may be detached from the instrument. Obtain the infrared spectrum after calibrating the instrument using a polystyrene form. For motor oils, use a cell. Obtain spectra also for reference and industrial comparison samples.

C Ratios of Infrared Absorbance

Measure the infrared absorbances at the following frequencies:

3050^{cm-1}, 2925^{cm-1}, 1600^{cm-1},

1375^{cm-1}, 810^{cm-1}, 720^{cm-1}

Determine the length of the vertical line, which intersects the line tangential to the two proximate inflections. Consider the ratio of intensity of the infrared absorption at one frequency to that intensity of the absorption at another frequency. Calculate the following ratios and compare with those for the products listed in Table 3.

TABLE 2

TABLE OF DENSITY PROPERTIES OF PETROLEUM PRODUCTS

Material	Density	API Gravity
Crude	0.857 - 0.976	13.5 - 33.5
High gravity naphtha	0.684 - 0.802	45 - 75
Low gravity naphtha	0.868 - 0.877	30 - 53
Gasoline	0.732 - 0.747	58 - 62
Jet fuel	0.759 - 0.825	40 - 55
Kerosene	0.797 - 0.825	40 - 46
Gas oil	0.859 - 0.877	30 - 33
White oil	0.866 - 0.882	29 - 32
Motor oil	0.877 - 0.909	24 - 30
Fuel oil #1	>0.849	>35
Fuel oil #2	>0.898	>26
Fuel oil #4	0.844 - 1.006	9 - 36
Fuel oil #5	0.909 - 1.025	6.5 - 24
Fuel oil #6	0.917 - 1.101	-3 to 23
Asphalt	0.947 - 1.147	-8 - 18

Establish the characterization of the petroleum product by comparative evaluation of the two ratios.

$$\frac{810^{cm-1}}{1375^{cm-1}} \text{ versus } \frac{810^{cm-1}}{720^{cm-1}}$$

Plot the ratio values on graph paper similar to Figure 1 and determine from the commercial asphalt-fuel oil line, $y = 0.13x + 0.13$, whether the point of the unknown lies in the asphaltic of in the heavy residual oil classification. Make a similar examination of the reference sample that will be compared to the unknown.

Asphalts are represented by values clustered between 0.6 and 1.3 for the $\frac{810^{cm-1}}{720^{cm-1}}$ ratio while heavy residual oils lie at points greater than 1.30 for this ratio.

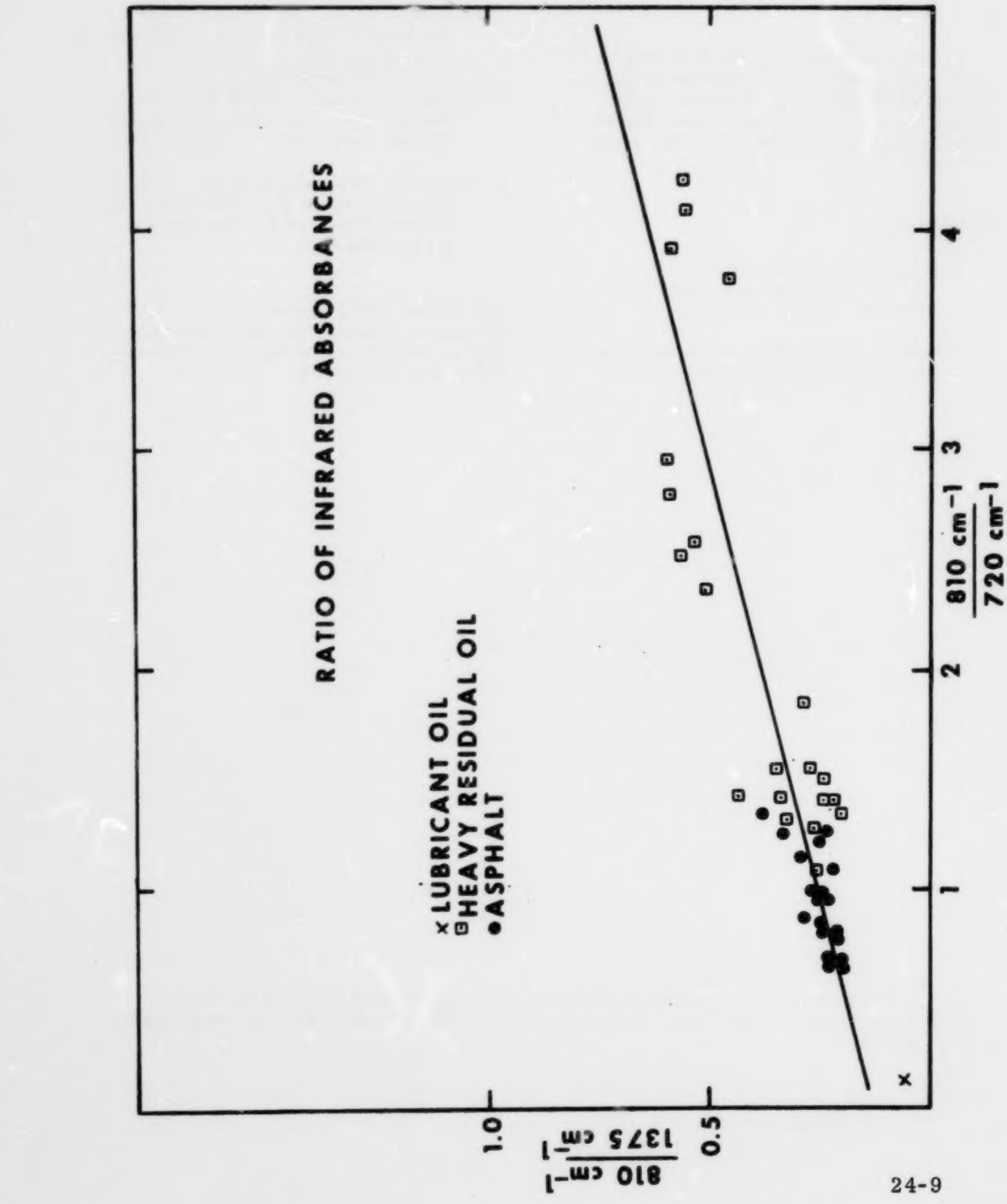
IV IDENTIFICATION

For identification purposes, determine whether the spectra of the collected oil slick and the suspect oil match along the entire spectral region between 2.5 μ and 15 μ. A match will establish the responsible party.

TABLE 3

RATIOS OF INFRARED ABSORBENCES OF COMMERCIAL ASPHALTS, NO. 6 FUEL OILS, AND LUBE OILS

RIA	Asphalt	No. 6 Fuel Oil	Lube Oil
$\frac{720^{cm-1}}{1375^{cm-1}}$	0.28	0.20	0.48
$\frac{3050^{cm-1}}{2925^{cm-1}}$	0.22	0.28	0.20
$\frac{810^{cm-1}}{1375^{cm-1}}$	0.25	0.40	0.06
$\frac{810^{cm-1}}{720^{cm-1}}$	0.94	2.18	0.12
$\frac{1600^{cm-1}}{1375^{cm-1}}$	0.54	0.60	0.11
$\frac{1600^{cm-1}}{720^{cm-1}}$	2.05	3.27	0.24


RATIO OF INFRARED ABSORBANCES
× LUBRICANT OIL
⊡ HEAVY RESIDUAL OIL
● ASPHALT
1
2
3
4
810 cm⁻¹ / 720 cm⁻¹
1.0
0.5
810 cm⁻¹ / 1375 cm⁻¹

V ERROR

The error is estimated to be about ± 10%. Deviations may be greatly minimized by averaging the data from four spectra at thickness of oil adjusted so that the transmission reading is 85% to 98% of full scale at $3050^{cm^{-1}}$.

REFERENCES

1 Johnson, W.D. et al. Proc. 11th Conference Great Lakes Research. 550-564. 1968.

2 Kawahara, F.K. Environmental Science and Technology 3, 150-153. 1969.

3 Kawahara, F.K. Laboratory Guide for the Identification of Petroleum Products. U.S. Department of the Interior, Federal Water Pollution Control Administration, Division of Water Quality Research, Analytical Quality Control Laboratory. January 1969.

4 Kawahara, F.K. and Ballinger, D.G. Industrial and Engineering Chemistry, Product Research and Development. (to be published)

This outline was prepared by F. K. Kawahara, Organic Chemist, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

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*Mention of the products and manufacturers is for identification only and does not imply endorsement by the Federal Water Pollution Control Administration of the U.S. Department of the Interior.

INFRARED INSTRUMENTATION

I THEORY

A The Effect of Infrared Radiation on the Molecule

1 All molecules are made up of atoms connected by chemical bonds which act very much like springs.

2 Atoms or atomic groups are in continuous motion with respect to each other. Each molecule has specific vibrational frequencies for these motions.

3 A molecule which has the same vibrational frequency as the infrared radiation striking it will absorb that energy.

4 If the radiant frequency differs from the characteristic frequencies of the molecule, the radiation passes through the molecule undiminished.

5 The characteristic frequencies for particular molecules are determined by the masses of their atoms, their spatial geometry and the strength of their connecting bonds.

B Measuring Infrared Radiation

1 The infrared spectrum gives basic information about the molecular structure of a compound.

2 Organic chemicals are largely made up of different combinations of atomic building blocks called functional groups: $-OH$, $-NH_2$, $-CH_3$, $-CO$, $-CN$, $-C-O-C-$, $-COOH$.

3 These subgroups have characteristic absorption bands in certain parts of the infrared spectrum.

4 Spectra correlation charts provide a key to the location of characteristic absorption bands of the most common functional groups.

II THE INSTRUMENT

A Source

1 A length of ceramic tubing heated to about 1200°C

2 It produces a continuous spectrum of radiant energy, most of which falls within 2.5 to 15 micron region.

B Monochromator

1 It disperses the radiation into its component wavelengths.

2 Selects the particular wavelength interval transmitted to the detector.

3 Maintains, by varying slit widths, approximately constant energy through the slits to the detector.

C Wavelength

1 A mirrors rotational position determines the particular wavelength which emerges from the slit.

2 Linkage with the recorder drum shaft insures that the wavelength scale on the drum corresponds exactly to the wavelength selected.

D Resolution

1 A NaCl prism provides the best combination of dispersion and transmission in the operating range.

2 The sealed and desiccated monochromator protects the prism from moisture and dust.

E Thermocouple

1 A high sensitivity thermocouple consists of a blackened gold leaf target welded to two pins.

2 Temperature differences at the junctions of the leaf and pins set up a thermoelectric potential which is utilized.

F Optical Attenuator

1 By substracting energy from the reference beam, the attenuator makes the amount of energy in the reference beam precisely the same as that in the sample beam.

2 This permits the pen to record directly in terms of sample transmittance.

G Instrument Performance

1 Scanning time

Twelve minutes for total scan, automatically programmed for constant rate per spectral slit width for constant accuracy over the wavelength range.

2 Accuracy

a Abscissa ± 0.03μ, ordinate ± 1%T.

b Less than 3% scattered light at 14.7μ and less than 1% from 2.5μ to 14μ.

III MICRO INFRARED TECHNIQUE

A Solution Technique

1 The technique most reliable for quantitative measurements in infrared region is the solution technique.

2 One drawback to this technique is the scarcity of suitable solvents with sufficient areas of transparency.

3 Carbon disulfide CS_2 is the most valuable of these because of its transparency in the "finger print" region of the infrared from 1,350 to 650 cm^{-1} (7.4μ to 15). Carbon tetrachloride is a useful solvent from 4,000 to 1,650 cm^{-1} (2.5μ to 6.0μ).

4 The sample is dissolved in 0.3 ml of solvent. The solution is then transferred to a 5 mm energy-path cavity cell by a hypodermic syringe. A matching cavity cell is used for solvent compensation.

5 With this technique, sensitivities down to 5-10 μg of strongly absorbing compounds are achieved without resorting to scale expansion.

B The Potassium Bromide KBr Pellet Technique

1 Since KBr is transparent throughout the commonly used portion of the infrared region, the entire infrared region is available as contrasted to the use of solvents.

2 A 6X beam-condenser is used to increase the energy transmitted through the pellet.

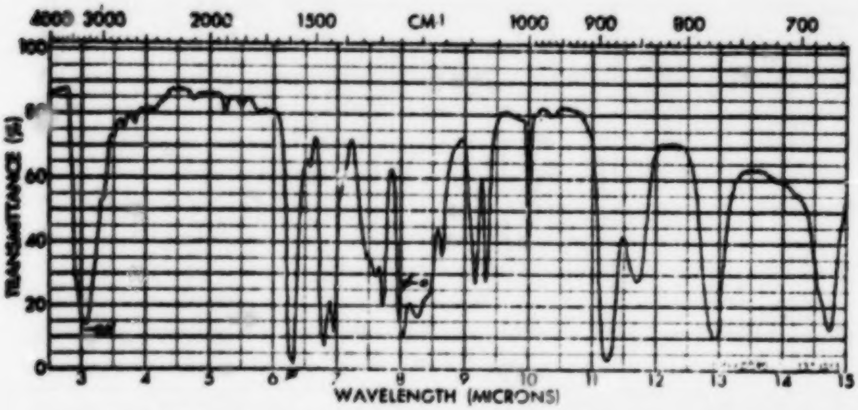
3 By this procedure a reliable sensitivity of about 5μg can easily be attained without scale expansion.

REFERENCES

1 Blinn, R.C. Infrared technique useful in residue chemistry, AOAC. Vol. 48, 1009-1017. 1965.

2 InfraCord, Perkin-Elmer spectrophotometer model 137-B Brochure, 1965.

This outline was prepared by J.W. Mandia, Chemist, formerly with FWQA, National Training Center, Cincinnati, OH 45226.



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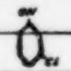
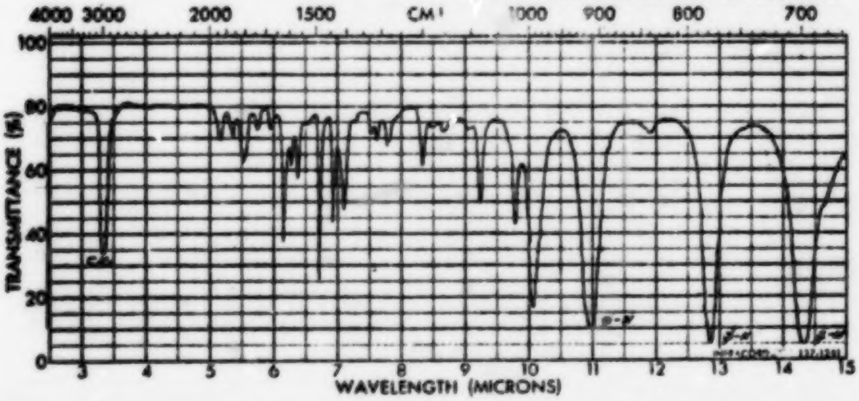
SAMPLE		1.	
M-CHLOROPHENOL	PURITY ACS	2.	
	PHASE LIQUID	DATE 3-24-58	
	THICKNESS SANDWICH	OPERATOR MGB	

SPECTRUM NO.___
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



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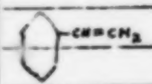
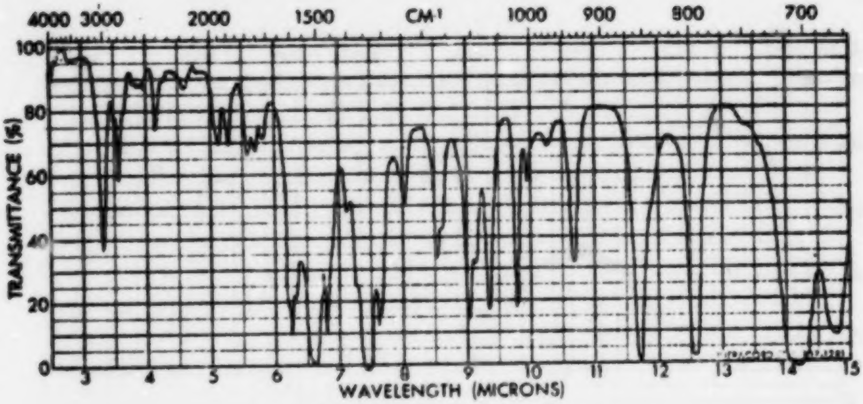
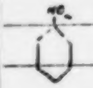
SAMPLE		1.	
STYRENE	PURITY ??	2.	
	PHASE LIQUID	DATE 4-14-59	
	THICKNESS SANDWICH	OPERATOR MGB	

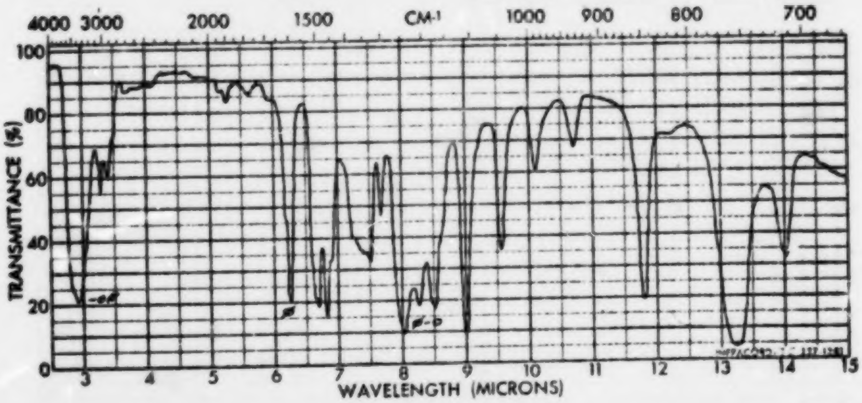
SPECTRUM NO.___
SAMPLE

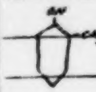
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 9	ORIGIN	LEGEND	REMARKS	SPECTRUM NO. SAMPLE
SAMPLE		1.		
NITROBENZENE	PURITY ACS	2.		
	PHASE LIQUID	DATE 3-4-59		
	THICKNESS 0.25 MM	OPERATOR DGB		

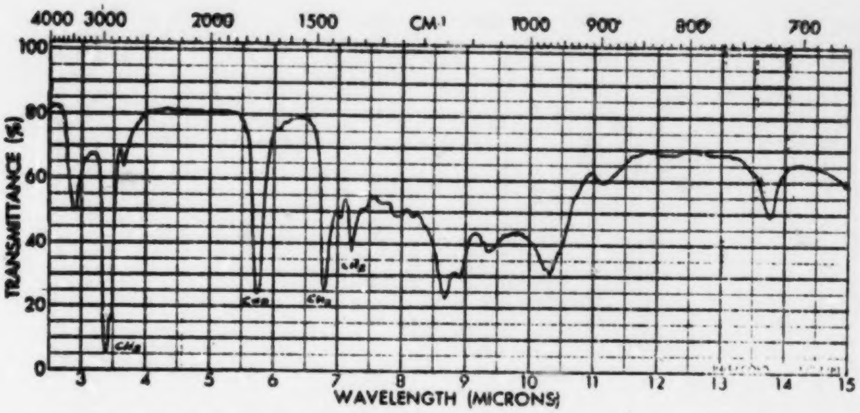
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 10	ORIGIN	LEGEND	REMARKS	SPECTRUM NO. SAMPLE
SAMPLE		1.		
O-CRESOL	PURITY ACS	2.		
	PHASE LIQUID	DATE 3-10-59		
	THICKNESS 0.2 MM.	OPERATOR DGB		

GR.OTS.Lab.5.4.59

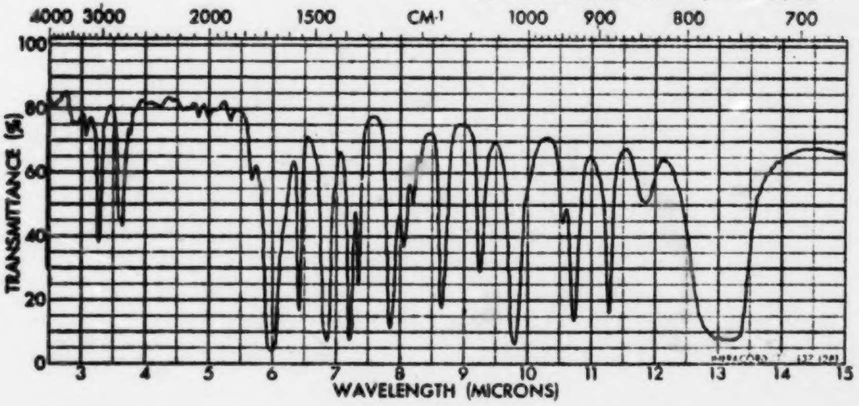
THE PERKIN-ELMER CORPORATION, NORWALK, CONN

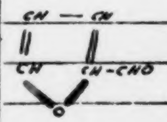


SPECTRUM NO: 7	ORIGIN	LEGEND	REMARKS
SAMPLE			
OCTANALDEHYDE	PURITY ??	2.	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CHO$
	PHASE Liquid	DATE 4-13-59	
	THICKNESS SNDWCH	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

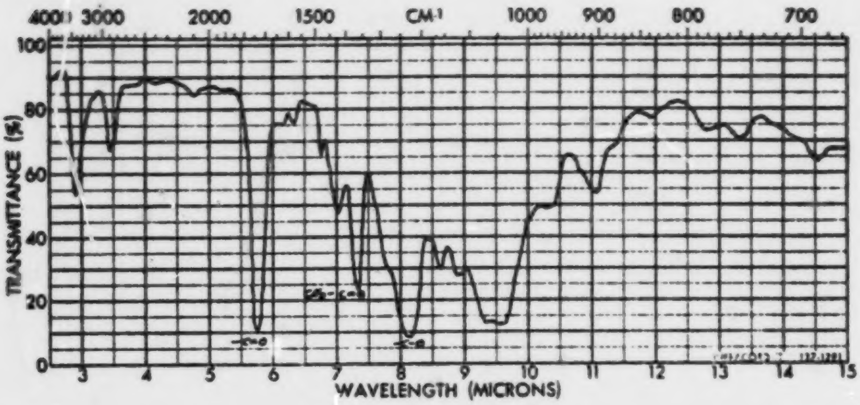


SPECTRUM NO. 8	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Furfural	PURITY ??	2.	
	PHASE Liquid	DATE 3/23/59	
	THICKNESS SNDWCH	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

GH.(95.1ab.b.b.59

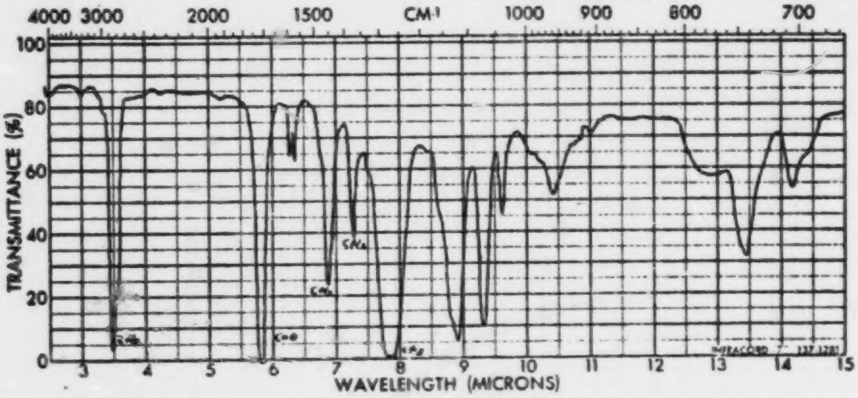
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

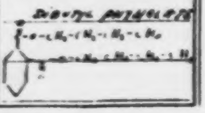


SPECTRUM NO. 5	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Acetate	PURITY ??	2.	$CH_3-\overset{O}{\overset{\|}{C}}-O-$
	PHASE Gel	DATE 4-1-59	
	THICKNESS Smear	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

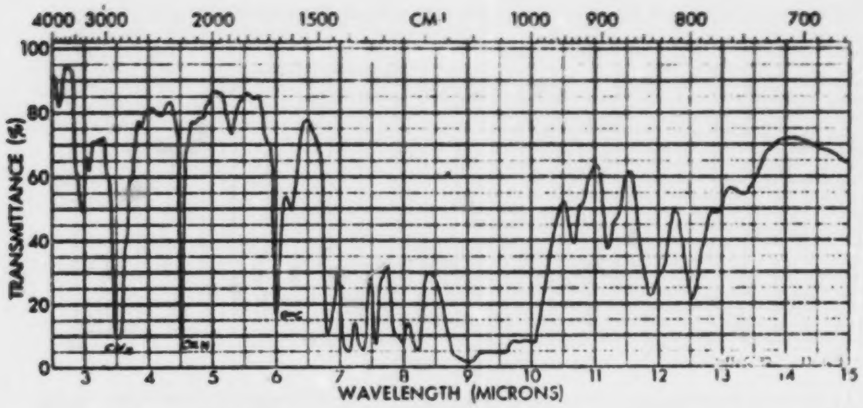


SPECTRUM NO. 6	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	Principally
Tygon Plasticizer	PURITY ??	2.	Dibutyl Phthalate 
	PHASE Gel	DATE 8-6-59	
	THICKNESS Smear	OPERATOR CGB	

SPECTRUM NO.
SAMPLE

CH.CES.lab.3.6.59

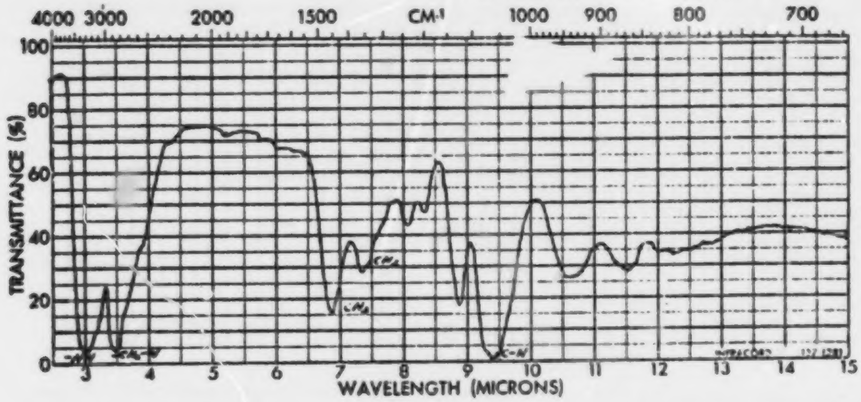
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 3	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
oxydipropionitrile	PURITY ACS	2.	NC-CH₂-CH₂ O CH₂-CH₂-CN
	PHASE Liquid	DATE 4-3-59	
	THICKNESS 0.2 mm	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

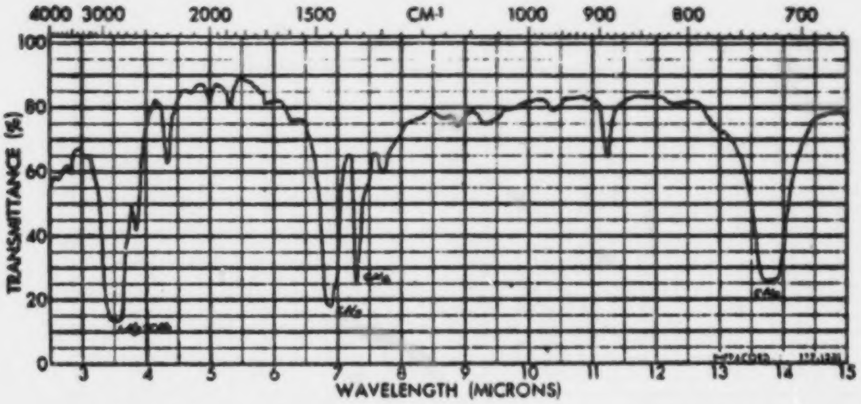


SPECTRUM NO. 89	ORIGIN	LEGEND	REMARKS
SAMPLE (4)		1.	
Diethanolamine	PURITY ACS	2.	HO-CH₂-CH₂ NH CH₂-CH₂-OH
	PHASE Liquid	DATE 4-2-59	
	THICKNESS sandwich	OPERATOR DGB	

SPECTRUM NO. 89
SAMPLE Diethanolamine

CR.OTS.1ab.7.4.59

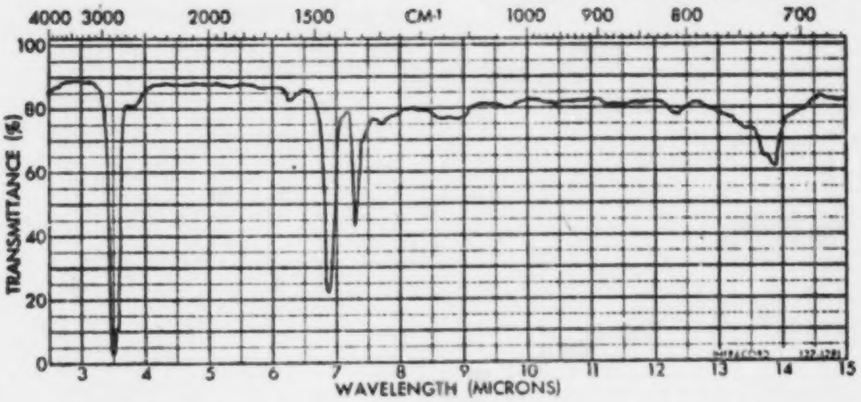
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 1	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Paraffin waxes	PURITY ??	2.	$-CH_2-CH_2-CH_2-$
	PHASE GEL	DATE 3-11-59	
	THICKNESS SMEAR	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

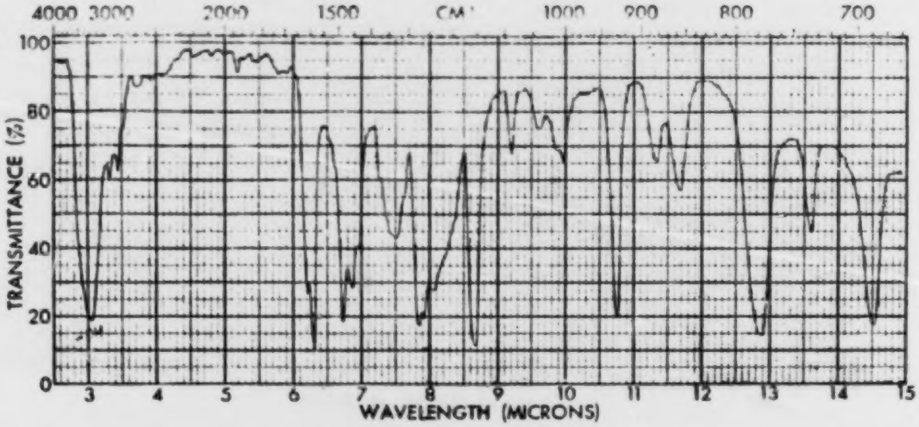


SPECTRUM NO. 2	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Petroleum residue	PURITY —	2.	
	PHASE GEL	DATE 2-11-59	
	THICKNESS SMEAR	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

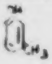
CR.OES.lab.1.6.59

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



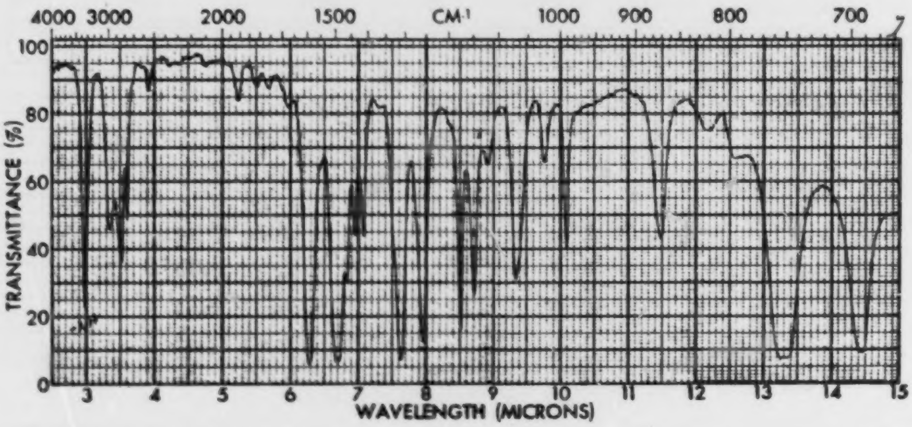
SAMPLE NO. 13
SOURCE PURE m-CRESOL
LOCATION
DATE SAMPLED
TYPE SANDWICH

REMARKS



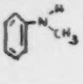
PHASE LIQUID
OPERATOR BAP
DATE 3-14-63

SAMPLE NO.
SOURCE
LOCATION



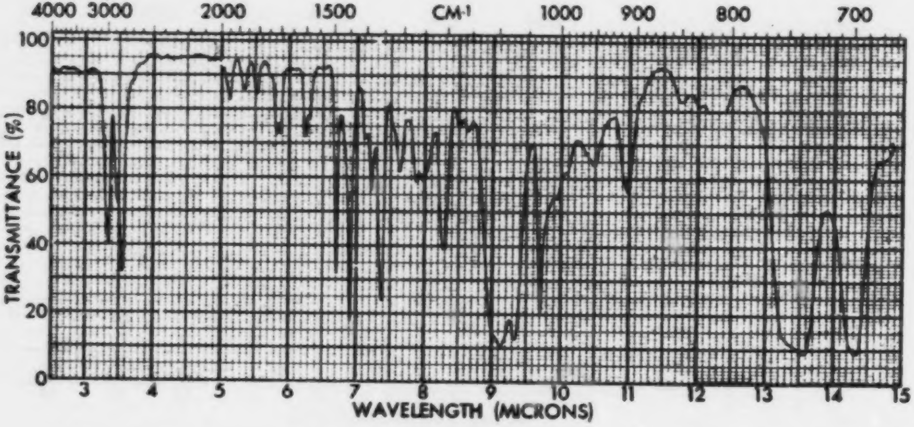
SAMPLE NO. 14
SOURCE N-METHYLANILINE
LOCATION
DATE SAMPLED
TYPE SANDWICH

PEMARKS



PHASE LIQUID
OPERATOR BAP
DATE 3-14-63

SAMPLE NO.
SOURCE
LOCATION



SAMPLE NO. 15

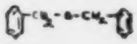
SOURCE PURE BENZYL ETHER

LOCATION

DATE SAMPLED

TYPE SANDWICH

REMARKS



PHASE LIQUID

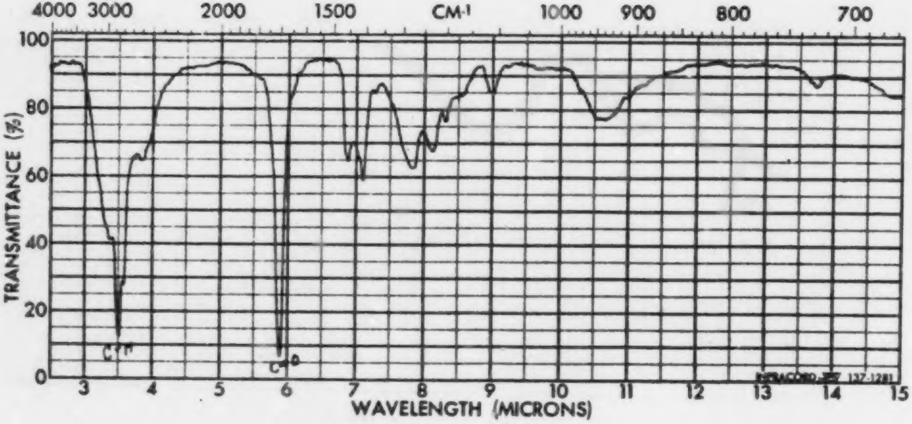
OPERATOR BAP

DATE 3-6-63

SAMPLE NO.

SOURCE

LOCATION



SPECTRUM NO. 16

SAMPLE CAPRYLIC ACID

ORIGIN

PURITY PURE

PHASE LIQUID

THICKNESS SMEAR

LEGEND

1.

2.

DATE 2-11-63

OPERATOR BAP

REMARKS

$CH_3CH_2CH_3CH_2CH_2CH_2CH_2C\begin{smallmatrix}{}^{\,O}\\{}_{OH}\end{smallmatrix}$

SPECTRUM NO.

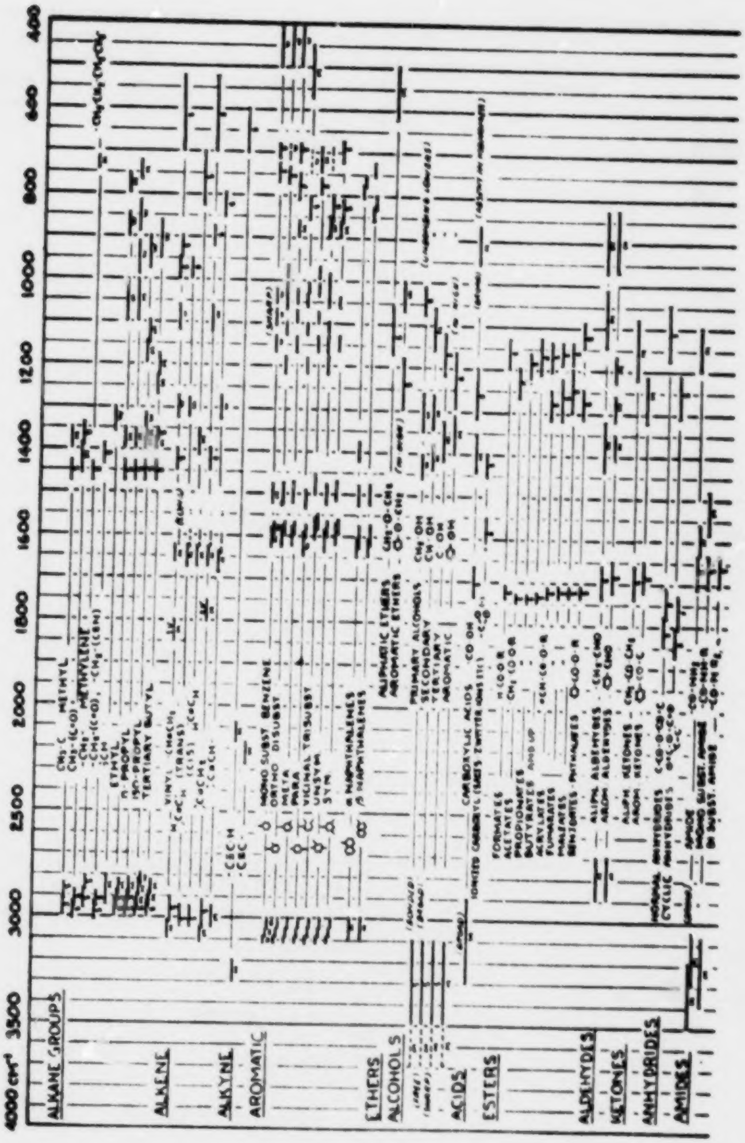
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

DATA SHEET - ORGANIC LABORATORY

SOLUBILITY SEPARATION		CHROMATOGRAPHIC SEPARATION OF NEUTRALS	
WEIGHTS	REMARKS	WEIGHTS	REMARKS
ORIGINAL SAMPLE __________ GM		ALIQUOT USED __________ __________ GM	
BASES __________ __________ GM		ALIPHATICS __________ __________ GM	
STRONG ACIDS __________ __________ GM		AROMATICS __________ __________ GM	
WEAK ACIDS __________ __________ GM		OXYS __________ __________ GM	
NEUTRALS __________ __________ GM		SUMMARY OF DATA BASES __________GM STRONG ACIDS __________ WEAK ACIDS __________ NEUTRALS __________ TOTAL RECOVERED __________GM % RECOVERY __________%	

N. B. COLTHUP



Reproduced from best available copy.

SPECTRA-STRUCTURE CORRELATIONS

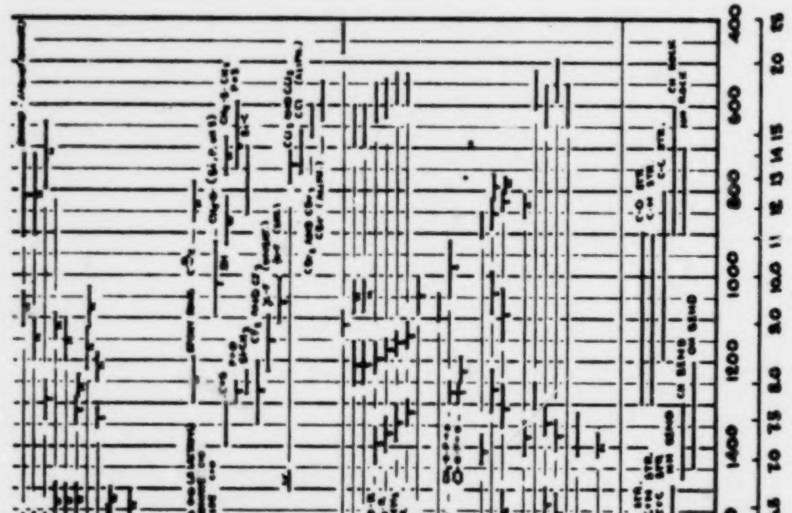


Chart 1. Probable positions of characteristic infra-red absorption bands.

Chart 1. From Colthup, N. B. "Spectra-Structure Correlations in the Infra-Red Region". Journal of the Optical Society of America. 40:397-400. June, 1950.

THE PROBLEM OF IDENTIFICATION IN THE ANALYSIS OF ORGANIC COMPOUNDS

I THE POLLUTION ROLE OF ORGANIC CHEMICALS

A Where they originate: Industries, biological (sewage, food industries), agriculture (pesticides), natural (vegetation, photosynthesis).

B Their adverse effects: Toxicity (pesticides), color, taste and odor (industrial and natural), fish tainting, foaming, fouling of ion-exchangers, carcinogenesis, teratogenesis.

C Why they must be identified: Discover the compound responsible for an observed effect, alert to unsuspected dangerous effects, find the responsible source, develop evidence to force abatement, monitor effectiveness of abatement measures.

II THE BACKGROUND OF IDENTIFICATION PROBLEMS

A Complexity of organic pollution: Multiple sources, many organics at each source, variability of operations, obscuration by natural processes in stream, low concentrations.

B Sampling problems: No right of entry to plant, inaccessible outfalls, use of concentrating procedures (adsorption and extraction).

C Special value of modern instruments: Carbon dating.

III PRINCIPLES OF CLASSICAL METHODS OF ORGANIC ANALYSIS

A Separation into individual substances by simple physical methods: Distillation, solubility separation.

B What elements are present: Elemental analysis, molecular weight.

C What is the molecular arrangement: Functional group analysis, preparation of derivatives, determination of specific properties (boiling point, melting point, density, refractive index, crystal form, color).

IV ELEMENTAL ANALYSIS OF ORGANIC CONTAMINANTS - Pesticide Analysis is Most Demanding Application

A Conventional methods require too much material.

B Special ultra-sensitive methods, qualitative and quantitative.

1 Coulometric detectors for: Cl, N, S, P.

2 Other GLC-type detectors: thermionic emission, Na or Cs for P; photoemission, plasma excitation or flame photometric.

3 Micro-combustion: CHN apparatus, O analysis, TOC.

4 Optical methods: flame photometric, atomic absorption, arc emission, X-ray fluorescence.

C Molecular Weight Determinations.

1 Indirectly from GLC: complex combination of boiling point, polarity, substrate properties.

2 Directly from mass spectrometry. Normal mode gives MW through mass of parent peak. High-resolution mode also gives elemental composition.

3 Osmometry

4 Gel permeation and diafiltration

V MOLECULAR CONFIGURATION OF ORGANIC CONTAMINANTS - Identification

A Functional groups - partial identification

1 Chemical reactions, e.g., for nitro group in parathion, double bonds by Br_2, phenols

2 Acid and base groups used in group separation

3 Instrumental methods: IR and NMR

B Specific molecular properties

1 Information incidental to isolation: adsorption (TLC or column), liquid partition, solubilities, steam volatility, odor, toxicity

2 P-values, counter-current distribution

3 Chromatographic constants - gas and liquid

4 Carbon skeleton analysis

5 Threshold values and TLM

C Instrumental fingerprints

1 UV-Vis-fluorescent spectrophotometry

a Activation-emission spectra

b pH shifts

2 Infrared

a Functional groups

b Fingerprints (2500 information bits, vs. GLC)

3 Mass spectra - fragmentation patterns

4 NMR - emphasis on electronic environment of hydrogen atoms

a Both functional groups and fingerprints

5 Induced electron emission spectrometry

VI APPLICATIONS OF IDENTIFICATION DATA

A Planning and motivating pollution abatement

B Legal evidence

This outline was prepared by A. A. Rosen, Chief, Waste Identification and Analysis Activities, Advanced Waste Treatment Research Laboratory, WQO, Cincinnati, OH 45226.

ANALYSIS OF FISH TISSUE
FOR ORGANO CHLORINE COMPOUNDS

I OBJECTIVE

Upon completion of this laboratory, the trainee will be able to extract, partition, cleanup and identify organo chlorine compounds in fish tissue. The student should be able to repeat the procedure in his own laboratory by using the printed laboratory procedure.

II REAGENTS

A Hexane, Burdick and Jackson, BP 68-69° C (redistilled)

B Acetonitrile, Burdick and Jackson, BP 80 - 2° C (redistill with 1 ml 85% H_3PO_4 per gallon).

C Ethyl ether, anhydrous, reagent grade

D Alumina, Fisher A-540

E Celite 545 Johns-Manville

III SPECIAL EQUIPMENT

Sorvall Omni-mixer

IV SAMPLE PREPARATION

The fish should be preserved by freezing as soon as possible after collection. When time permits, it may be skinned and a filet portion selected and cut into 10 or 20 gm portions for extraction. Refreeze these samples.

V EXTRACTION

A Cut the frozen sample of fish tissue quickly into small pieces (roughly 1 cm cubes). Place about 20 gm of the tissue into the Omni-mixer (or Waring Blender, explosion proof) with 175 ml of hexane and about 9-10 gm of Celite 545. Homogenize for about 30 seconds. Remove the stainless steel container and hold under a hot water tap for 1 minute; replace on the mixer and homogenize for 2.5 minutes.

B Decant the supernatant liquid, and mix the tissue with another 175 ml portion of hexane for 3 minutes.

C Decant, combine the extracts, and filter with suction through Whatman #1 paper in a Buchner funnel.

D Evaporate the extract to about 10 ml.

VI HEXANE-ACETONITRILE PARTITION

A Transfer the extract to a 125 ml separatory funnel and bring to a 25 ml volume with hexane. Mark the separatory funnel at the 25 ml mark.

B Add 100 ml of acetonitrile to a 250 ml Erlenmeyer flask (CAUTION: Acetonitrile is extremely toxic; avoid contact with skin). Saturate the acetonitrile with hexane by adding hexane until the solution appears and remains cloudy for a few seconds after swirling (approximately 15 ml of hexane is required).

C To the separatory funnel add 25 ml of acetonitrile saturated with hexane. Gently mix solvents in separatory funnel by rocking back and forth a few times (CAUTION: Release pressure in separatory funnel by opening stopcock).

D Extract pesticides into acetonitrile by gently rocking separatory funnel back and forth for 30 seconds. (NOTE: Severe emulsions may occur. Emulsion will begin to break if allowed to stand for a few minutes. PATIENCE!)

E Drain lower layer (acetonitrile) into Kuderna-Danish evaporator.

F Re-extract with 3 additional 25 ml portions of redistilled acetonitrile saturated with hexane. Collect all acetonitrile extracts in Kuderna-Danish evaporator. Discard hexane fraction.

G Evaporate acetonitrile extract to approximately 10 ml. Evaporate to dryness in a hot water bath under a gentle stream of filtered air. (NOTE: The residue may be a yellow oil if the fish sample has a high fat content. In this case, evaporate until a clear oil is produced.) Take up the residue in 10 ml hexane.

VII COLUMN ADSORPTION CHROMATOGRAPHY

A Prepare slightly deactivated adsorption alumina (1.4%) by adding 0.7 ml distilled water dropwise with a pipette on top of 50 gm of alumina (Fisher A-540) in a small glass jar. Distribute drops over entire surface of alumina. Using a stirring rod mix and break up the lumps formed in the alumina. Cap the jar and tumble gently for 3 minutes to mix the alumina.

B Into a chromatographic column place about 16 cm of deactivated alumina. Tamp to 15 cm with a rubber stopper.

C Place a 50 ml graduate under the column; with another 50 ml graduate add 50 ml hexane (redistilled) to column to prewet it. Just after adding 50 ml of hexane, mark 50 ml level on column with a grease pencil. Adjust elution rate to 3.5 to 4.0 ml per minute (approximately 1 drop per second). When hexane has just reached level of alumina, using a stirring rod transfer the hexane sample extract to the column. Wash sample tube with additional 10 ml of hexane.

D When hexane has just reached level of column, add 50 ml eluting solution (9:1 hexane/ethyl ether). Level of eluting solution in column should correspond to first 50 ml mark. If it does not, remark column at new level. Place a 125 ml Erlenmeyer flask under the column and collect the first 50 ml fraction (mark on a separate sheet of paper the time at which elution begins). Throughout the collection of the fraction, keep the level of the eluting solution at the 50 ml mark on the column; this is important in order that the elution rate of 3.5-4.0 ml/min be maintained. After the first 50 ml fraction has been collected, again mark the time. If elution rate is adjusted properly, collection of the fraction should take 12-14 minutes. Increase or decrease elution rate as required. Begin immediately to collect the second 50 ml fraction. Proceed as in the collection of the first fraction. Collect a total of 5 fractions. Cap with aluminum foil and identify each flask.

VIII ELECTRON CAPTURE GAS CHROMATOGRAPHY

A Prepare a standard curve for the pesticide standards at your work bench by using a gas chromatograph equipped with an electron capture detector.

B Inject 5-10 µl of each fraction (from the alumina column) into the gas chromatograph. Tentatively identify and quantitate the insecticide in each fraction.

C Composite the fractions containing the pesticides and concentrate to 5 ml in a Kuderna-Danish evaporator. Further concentrate to 1-2 ml in a hot water bath with a gentle stream of filtered air. Transfer to a chromatographic storage tube. Further concentrate to 0.2 ml in a hot water bath with a gentle stream of filtered air. When extract reaches 0.2 ml immediately cool in a cold water bath. Rinse walls of tube with 100 µl hexane and concentrate to 0.1 ml in a hot water bath with a gentle stream of filtered air. When extract reaches 0.1 ml immediately cool in a cold water bath and store in the refrigerator until ready for TLC. (NOTE: The heating and cooling steps are essential in preventing losses due to the oily extract creeping up the walls of the tube.)

IX THIN LAYER CHROMATOGRAPHY

A Spotting of Plate

1 Lay a kimwipe open on top of bench. Scrape 1/4 inch area on two sides of plain Al_2O_3. Position plate on kimwipe.

2 Place a kimwipe folded 1/4 size on left hand side of the work area.

3 Place DDE and γ chlordane standard vials (1 μg/μl) and tube containing gamma chlordane fish fraction (in cool water bath) along side of folded kimwipe.

4 Place a template with graduated edge upon the aluminum oxide plate with starting line about one (1) inch from bottom.

5 Mark with a pencil the starting line and 10 cm line on both sides of plate.

6 Insert (5 μl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

7 Rinse capillary twice with DDE removing contents each time on kimwipe.

8 At spotting position #1 of template, touch capillary filled with DDE once lightly on plate removing approximately 1/4 of contents of capillary.

9 At position #2 spot two times putting 1/2 the contents of the capillary on the plate. Permit each spot to dry before respotting.

10 Remove contents of the capillary on kimwipe and refill from vial.

11 At position #3 spot the contents of the capillary (5 μl) by lightly touching the capillary to the plate removing about 1/4 of the contents with each spotting. Permit the spots to dry before respotting.

12 Rinse capillary 5 times in hexane.

13 At positions #4 - #6, spot gamma chlordane. Repeat spotting procedure as described in items 8 - 12.

14 Rinse 20 μl capillary in hexane.

15 Insert 20 μl capillary into chromatographic storage tube containing sample. Allow capillary to draw up sample by capillary action only (approximately 0.01 ml). At position #7, spot the contents of the capillary. Refill the capillary and spot again. Continue refilling and spotting until entire sample has been spotted. Always allow spots to dry before respotting.

B Development in Solvent

1 Place plate, coated surface facing solvent saturated paper, in developing tank containing 1% ethyl ether in hexane with marked spotting line down. Develop to 10 cm line.

2 Lift off top of jar and remove plate, lifting plate with finger tips of both hands. Dry in air for 10 minutes.

3 Place plate in hood and spray with Rhodamine B. The spray should be a fine mist giving an even, smooth light pink color to surface of plate.

4 Place plate in dark and observe purple spots with UV light.

5 Measure distance (cm) of spotting line to solvent front. Measure distance of spots from starting line.

6 Calculate R_f of spots which appear in fish sample.

$$R_f = \frac{\text{Distance of spot}}{\text{Distance of solvent front}}$$

C Preparation of Thin-Layered Separated Pesticides for Infrared Spectrophotometry

1 Marking off spot area on plate

a Place template below lower spot line. Mark surface with spatula across plate.

b Move template above these spots and similarly mark surface.

c Move template to a line above the gamma chlordane spots and mark surface. Similarly, mark surface below these spots.

d Turn template perpendicular to selected spot; mark surface across plate.

e Move template to other side of spot and mark surface.

2 Removal of spot from plate

a Insert a tuft of glass wool and position at the tapered end of medicine dropper.

b Mark the medicine dropper to identify the pesticide spot collected.

c Connect vacuum tubing over tapered end of medicine dropper.

d Collect all of adsorbent surface enclosed between boundaries of marked off area by sucking the adsorbent and pesticide by vacuum and depositing in glass wool of medicine dropper.

3 Elution of pesticide

a Mark a 15 ml centrifuge tube.

b Place paper clip over tapered end of medicine dropper approximately 2/3 distance from large end.

c Insert tapered end of dropper into tube supported by paper clip.

d Elute pesticide with 5 ml of 4:1 hexane-ether mixture into 15 ml centrifuge tube.

e Concentrate to 1-2 ml in hot water bath with a gentle stream of filtered air. Transfer to chromatographic storage tube. Further concentrate to 0.2 ml in a hot water bath with a gentle stream of filtered air. When eluate has reached 0.2 ml, immediately cool in a cold water bath. Wash walls of tube with 100 µl hexane. Concentrate to 0.2 ml in a hot water bath with a stream of filtered air. Stopper and store in refrigerator or cool water bath for IR.

X NOTES ON PROCEDURE

A All solvents must be of the highest quality. Even solvents which can be purchased in a redistilled form (e.g. Burdick and Jackson) must be redistilled again in the laboratory.

B The analyst should be careful throughout the procedure to avoid unnecessary loss of pesticide. This can be done by care in evaporation, use of tapered centrifuge tubes for transfer and care in spotting the thin-layer plate.

C Table 1 shows the separation of chlorinated hydrocarbons which should be expected with this column. It may be necessary to increase or decrease the amount of water used for deactivation of the alumina in order to achieve the proper separation.

This outline was revised by W. E. Loy, Chemist, Analytical Services and W. R. Davis, Chemist, Training Officer, Southeast Water Laboratory, Athens, GA.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

I INTRODUCTION

Atomic absorption spectroscopy has been well known to physicists and astronomers for more than 100 years. In 1850, Kirchoff took light from the sun and collimated it with a lens through the flame of an ordinary laboratory burner, and then passed the light through a prism which dispersed it into the characteristic visible spectrum with which we are all familiar. He then took a platinum spoon containing a sodium salt and introduced it into the flame. He observed that the yellow light that was present in the spectrum disappeared and in its place appeared the characteristic resonance lines of sodium. Since then astronomers have used the technique to detect and measure the concentration of metals in the vapors of stars. In 1953, Walsh(1) recognized its potential advantage over emission spectroscopy for trace metal analysis. He designed and built an analytically useful atomic absorption instrument. Shortly thereafter the advantages of atomic absorption instrumentation were recognized in the United States.

II THEORY

The basis of the method is the measurement of the light absorbed at the wavelength of a resonance line by the unexcited atoms of the element. Elements not themselves excited to emission by a flame may be determined in a flame by absorption provided that the atomic state is capable of existence.

At the temperature of a normal air-acetylene flame (2100°C) only about one per cent of all atoms is excited to emission in a flame; therefore absorption due to a transition from the ground electronic state to a higher energy level is virtually an absolute measure of the number of atoms in the flame, and the concentration of the element in the sample. Electrons will absorb energy at the same characteristic wavelength at which they emit energy. This is the principle upon which the technique of atomic absorption spectroscopy is based.

The advantages of atomic absorption spectroscopy as compared to emission spectroscopy are: (1) that atomic absorption is independent of the excitation potential of the transition involved and (2) that it is less subject to temperature variation and interference from extraneous radiation and interference from extraneous radiation or energy exchange between atoms.

Atomic absorption analytical apparatus (Figure 1) consists of a suitable source of light emitting the line spectrum of an element, a device for vaporizing the sample, a means of line isolation (monochomator or filter) and photoelectric detecting and measuring equipment.

If the detector is placed to receive only the resonance line of the element from the light source, measurement can be made of the absorption of resonance-line radiation on its passage through the vaporized sample. The magnitude of this absorption gives a measure of the concentration of free ground-state atoms of the element in the vapor and when referred to a calibration curve, provides a means of determining the concentration of the element in the sample.

III INSTRUMENTATION

The general arrangement of an absorption flame photometer is no different from an emission flame photometer except for the addition of a light source. An aerosol is introduced into a flame which is placed on the optical axis between the entrance slit of the monochromator and the monochromatic light source. Energy of the wavelength absorbed by the sample is provided by a source lamp whose emitting cathode is made of that element. This energy is passed through the flame and then through a dispersing device. A detector measures the absorbed and unabsorbed exciting radiation.

A Light Source

For the more volatile elements such as the alkali metals, mercury and thallium,

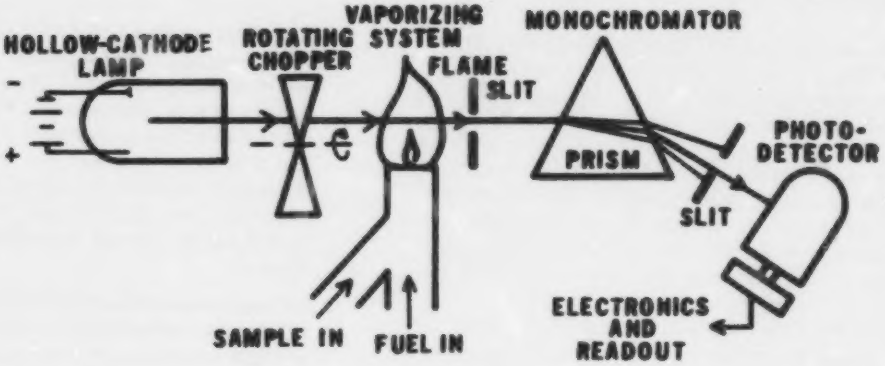

HOLLOW-CATHODE LAMP
ROTATING CHOPPER
VAPORIZING SYSTEM
FLAME
SLIT
MONOCHROMATOR
PRISM
PHOTO-DETECTOR
SLIT
ELECTRONICS AND READOUT
SAMPLE IN
FUEL IN
-
+

FIGURE 1

the most convenient source is the spectral vapor lamp, which consists of a closed glass or silica tube, into which are sealed oxide-coated electrodes, containing one of the rare gases and some of the appropriate metals. For line sources of the less-volatile elements, hollow-cathode discharge tubes have been found the most satisfactory. These consist of an anode and hallow cylindrical cathode (either composed of or lined with the appropriate metal) mounted in a sealed glass tube containing one of the rare gases (helium or argon).

Lamps are operated at low currents to improve linearity of response and maintain narrow emission lines.

B Vaporization of Sample

1 Atomic-absorption methods have been applied almost exclusively to the analysis of solutions and for this purpose flames, fed with a fine spray of the sample solution, similar to those employed in flame photometry are used.

The burner has two principal functions to perform:

a It must introduce the sample into the flame.

b It must reduce the metal to the atomic state.

2 Burners can be classified as:

a Total-consumption

Those which introduce the sample spray directly into the flame. (Figure 2)

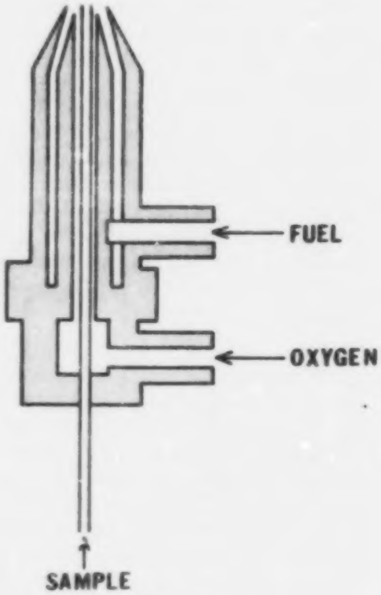

FUEL
OXYGEN
SAMPLE

FIGURE 2

b Premix

Those which introduce the spray into a condensing chamber for removing large droplets. (Figure 3)

3 Flame shape is important. The flame should have a long path length but a narrow width, such as a fishtail flame) so that the source traverses an increased number of atoms capable of contributing to the absorption signal.

4 The effective length of the flame may be increased by multiple passages through the flame with a reflecting mirror system, or by alignment of several burners in series.

5 The flame temperature need only be high enough to dissociate molecular compounds into the free metal atoms.

Typical flame temperatures are shown in Table I.

Table I

Fuel-Oxidant	Approximate Temp., °C
Nitrous Ozide - acetylene	3000
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen	4900

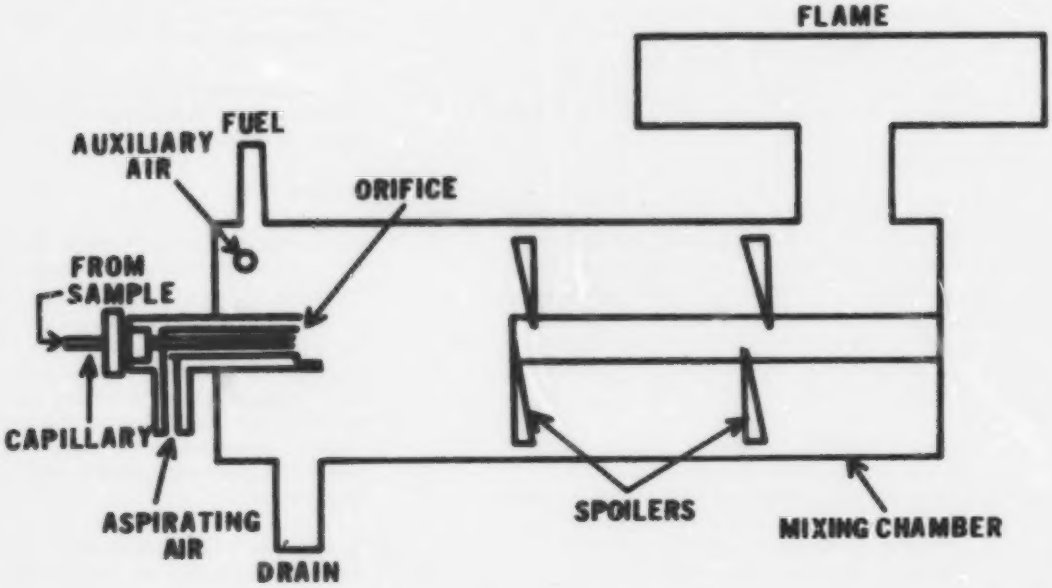


Figure 3

C Line Isolation

1 The use of a line spectrum of the element being determined, rather than a continuous spectrum, makes possible the use of monochromators of low resolving power or even filters. When a spectral lamp is used as a light source, it is only necessary to isolate the resonance line from neighboring lines of the light source or vaporized sample. The resolution of the method is implicit in the width of the emission and absorption lines.

2 To realize the full potentialities of the method, the strongest absorption line must be used. For elements with simple spectra, the resonance line arising from the lowest excited state is usually the line exhibiting strongest absorption.

3 Calibration curves depart from linearity at much lower concentrations in absorption work as compared with emission work. Curvature results partly from increased pressure broadening as the concentration of salt rises, but also depends on source characteristics, particularly self-absorption, and on the nature and homogeneity of the flame.

D Detection

1 Photo-electric detectors used in atomic absorption analysis need be no more sensitive than those used in emission analysis, since in the atomic absorption method, concentration of an element is determined by measuring the reduction in intensity of the resonance line emitted from a source of high intensity.

2 Single or double-beam circuits may be adopted for work with a single beam instrument, results are directly dependent upon source and detector stability. Both must be powered by separate power supplies. In a double-beam system small variations in the source signal are compensated automatically.

IV EVALUATION

A Sensitivity

1 For an air-acetylene flame of length 2 or 3 cm the lower limits of detection of elements having low resonance-line excitation potential (eg Na-K) are approximately equal in a single-beam atomic absorption and emission methods.

2 For elements having highly reversed resonance lines or resonance lines of high excitation potential, the atomic-absorption method has decided advantages over emission methods. Examples of elements in these categories are Zn, Mg, Fe and Mn.

3 A disadvantage of the atomic-absorption method, when compared with flame emission, is the lack of a quick and simple method of varying sensitivity to deal with solutions of widely varying element concentrations. The sensitivity of an atomic-absorption instrument is determined almost entirely by flame characteristics, notably length of light path through the flame.

4 A comparison of sensitivity obtained by emission and adsorption techniques is given in Table II.

B Precision

1 Precision of a single-beam atomic absorption instrument is primarily a function of the stability of light output from the spectral lamp. This in turn is dependent on the stability of the main supply and inherent stability of the lamp. The largest fluctuations are only ± 2 percent for the hollow cathode tube and sodium spectral vapor lamp. A double-beam instrument significantly reduces this error.

2 In common with flame-emission methods, atomic absorption is subject to "noise" from the flame and the detector. Changes in absorption caused by fluctuations in

Element	Sensitivity mg/l Flame	A. A
Aluminum	2	0.5
Antimony		0.2
Arsenic		1.0
Barium	0.3	1.0
Beryllium	25	0.05
Bismuth		0.2
Cadmium	2	0.01
Calcium	0.003	0.01
Cesium		0.05
Chromium	0.1	0.01
Cobalt		0.15
Copper	0.01	0.005
Gallium		1.0
Gold		0.1
Imdium		0.5
Iron	0.2	0.05
Lead	2	0.15
Lithium	0.002	0.005
Magnesium	0.1	0.003
Manganese	0.01	0.01
Mercury	10	0.5
Molybdenum		0.2
Nickel		0.05
Palladium		1.0
Platinum		0.5
Potassium	0.001	0.005
Rhodium		0.3
Rubidium		0.02
Selenium		1.0
Silver	0.05	0.02
Sodium	0.002	0.005
Strontium	0.01	0.02
Tellurium		0.5
Titanium		1.0
Thallium		0.2
Tin		2.0
Vanadium		0.5
Zinc	200	0.005

Table II

flame temperature are much less than those in emission because the strength of the absorption line is principally dependent on Doppler width whereas the intensity of emission from the flame is much more sensitive to temperature.

C Accuracy

This is shown by the types of interference found in flame emission and atomic absorption spectroscopy. There are three types:

1 Physical

Collision of atoms and electrons or atoms and molecules will transfer energy thus causing an enhancement or depression of analysis-line emission. This has a large effect on flame emission analysis but has only a negligible effect on atomic absorption.

2 Radiative

Light from elements other than the one being measured pass the line isolating device (monochromator or filter). This occurs in flame emission work, for example, the interference of calcium and magnesium in sodium determinations. This interference is also encountered in atomic absorption using a D.C. system but is very small because of the large signal from the hollow-cathode tube. Radiative interference is eliminated in an A.C. system.

3 Chemical

Emission from an element in the flame is depressed by the formation of compounds, which are not dissociated at flame temperatures. This also affects absorption because the formation of temperature - stable compounds in the flame causes proportionate reduction in the population of ground-state and excited atoms.

Investigations to date suggest chemical interference is confined, almost entirely, to the alkaline-earth elements and that calcium absorption is more subject to this interference than is magnesium absorption.

Typical calibration curves are shown in Figure 5.

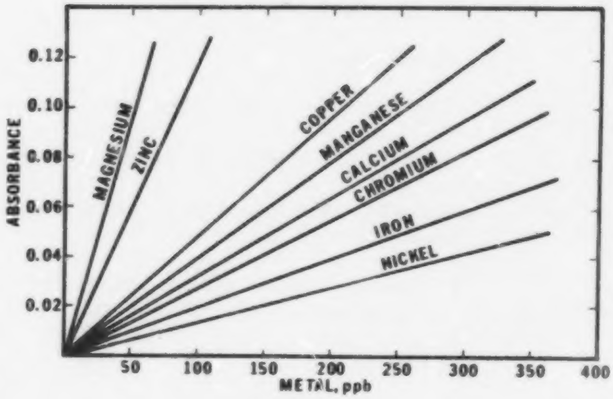


Figure 5

V REMOVAL OF INTERFERENCES AND CONCENTRATION OF SAMPLE

A Removal of Interferences

1 The methods for overcoming these interferences in atomic absorption are similar to those used in flame emission, namely, either separation of interfering ions or suppression of the interference by addition in excess of a substance that will prevent formation of compounds between interfering ions and the element being determined.

B Concentration of Sample

1 Organic separations can be used to concentrate a sample. Interferences are removed, as seen above, and also the organic solvent enhances the absorption.

2 Ion exchange has also been used successfully for concentrating samples for atomic absorption.

VI CONCLUSIONS

Atomic-absorption methods are as good as or better than emission methods, for elements to which they can both be applied, in sensitivity, precision and accuracy. They can be applied to a far wider range of elements than can emission analysis. The additional cost of hollow cathode discharge tubes is compensated by the greater range of analyses and greater reliability of results.

VII INSTRUMENTS AVAILABLE

A Perkin Elmer

1 Model 303 - double beam, AC -$5,920.00

2 Model 290 - single beam, AC -$2,900.00

B Beckman attachments for existing spectrophotometers.

1 Use with model D.U. and D.U. -2- single beam, DC - $2,135.00

2 Use with model D.B. - single beam, AC - $2,495.00

C Jarrell-Ash

1 Dual atomic absorption flame spectrometer - single beam, AC - $5,800.00

D E.E.L.

1 Atomic absorption spectrophotometer - single beam, AC $2,850.00.

ACKNOWLEDGEMENT:

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REFERENCES

1 Walsh, A. Spectrochim. Acta. 7, 108. 1955

2 Kahn, Herbert and Slavin, Walter. Atomic Absorption Analysis. International Science and Technology. November 1962.

3 David, D.J. The Application of Atomic Absorption to Chemical Analysis. The Analyses. 85:779-791. 1960.

4 Platte, J.A., and Marcy, V.M. A New Tool for Water Chemicals. Industrial Engineering. May 1965.

5 Biechler, D.G. Determination of Trace Copper, Lead, Zinc, Cadmium, Nickel, and Iron in Industrial Waste Water by Atomic Absorption Spectrophotometry After Ion Exchange on Dorvex A-1. Analytical Chemistry, 37:1054-1055. 1965.

6 Elwell, W.T., and Gidley, J.A.F. Atomic-absorption Spectrophotometry. The MacMillan Company. N.Y. 1962.

7 Willard, H.H., Merritt, L.L., and Dean, J.A. Instrumental Methods of Analysis. D. Van Nostrand Co., Inc. N.Y. 1965.

This outline was prepared by Paul F. Hallbach, Chemist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

METALS IN NATURAL AND TREATED WATERS IN THE ABSENCE OF GROSS POLLUTION BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Most metals can be determined by atomic absorption with an air-acetylene flame (see JAWWA. 60:739, 1968). A few, however, require a higher temperature flame to dissociate the molecules. Metals which can be determined by use of the higher temperature nitrous oxide-acetylene burner include aluminum, beryllium and barium. While barium can be determined by aspiration of the aqueous solution directly into the flame, the method is not sensitive enough to determine aluminum and beryllium at the concentrations of interest to water plant operators, unless concentrated by complexing with a chelating compound and extracting into an organic solvent.

I GENERAL DISCUSSION

A Principle

Atomic Absorption resembles emission flame photometry in that a sample is aspirated into a flame and atomized. The major difference rests in the fact that flame photometry measures the amount of light emitted, whereas in atomic absorption spectroscopy a light beam is directed through the flame, into a monochromator, and onto a detector which measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms and in the usual flames the ratio of unexcited to excited atoms at a given moment is very high. Since each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed for each element and hence the method is relatively free of spectral or radiation interferences. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

B Interference

The most troublesome type of interference is usually termed "chemical" and results from the lack of absorption from atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule or because the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. Barium fits into the former and aluminum and beryllium into the latter category and as a result, the nitrous oxide-acetylene flame is recommended because of its higher temperature. In addition, barium may undergo ionization in the flame, and the ground state (potentially absorbing) population is thereby reduced. This situation can be overcome by the addition of an excess of a cation having a similar or lower ionization potential.

II APPARATUS

A Atomic absorption spectrophotometer.

B Burner

Nitrous oxide burner head with 2 inch slot (reduces overheating frequently encountered with 3-inch slot). When using a nitrous oxide acetylene flame always remember to clean the burner slot frequently using a razor blade or equivalent prior to ignition to dislodge the carbon crust which forms along the slit surface approximately every 20 minutes.

C Separatory funnels

250 ml, preferably with teflon stopcocks, for extractions with organic solvents.

D Glassware

All glassware, including sample bottles, should be rinsed with 1 + 1 HNO_3 followed by deionized distilled water to avoid contamination errors.

III REAGENTS

A Deionized distilled water

Prepare by passing distilled water through a mixed bed of ion-exchange resins. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.

B Nitric acid, conc.

C Sodium chloride solution

Dissolve 250 grams of reagent grade sodium chloride in distilled water and make up to 1000 ml.

D Stock barium solution

Dissolve 1.7787 grams barium chloride ($BaCl_2 \cdot 2H_2O$) in 1000 ml distilled water to obtain a solution containing 1.0 mg/ml barium.

E Stock beryllium solution

Dissolve 20.7574 grams of beryllium nitrate, $Be(NO_3)_2 \cdot 3H_2O$, in 1000 ml distilled water to obtain a solution containing 1.0 mg/ml beryllium.

F Stock aluminum solution

Dissolve 8.792 g of potassium aluminum sulfate, $K_2Al_2(SO_4)_2 \cdot 24H_2O$ in distilled water and dilute to 1 liter in a volumetric flask to obtain a solution containing 0.5 mg/ml of aluminum.

G Standard solutions

In general, prepare a series of standard solutions containing 0.005 to 1.0 mg/l metal by appropriate dilution of the stock solution.

H Fuel and oxidant

Use nitrous oxide-acetylene flame for all the metals in Table I.

I Special reagents for determining low concentrations of aluminum and beryllium

1 8-Hydroxy-quinoline

Dissolve 20 grams in 1 N acetic acid (60 ml glacial acetic acid per liter) and make up to a volume of 1 liter with the 1 N acid.

2 Buffer

Dissolve 200 grams of ammonium acetate and 70 ml of concentrated ammonium hydroxide in distilled water and make up to a total volume of 1 liter.

3 Chloroform

IV PROCEDURE

A Pretreatment of sample

Acidify all water samples with nitric acid (1 ml concentrated nitric acid per 100 ml sample) and autoclave at 250°F for one hour to solubilize particulate matter, and readjust volume, if necessary.

B Precaution

Install the spectrophotometer on a level bench or table with the burner under a vent.

C Instrument operation

Because the differences between the makes and models of satisfactory atomic absorption spectrophotometers render impossible the formulation of detailed instructions applicable to every instrument, follow the manufacturer's operating instructions. As a rule, choose the correct hollow cathode lamp, install and align in the instrument; position the monochromator at the correct wavelength (Table I); select the proper monochromator slit width; set the light source current according to the manufacturer's recommendation; light the flame and regulate the flow of fuel and oxidant; adjust the burner for maximum percent absorption and stability; and balance the photometer. Then run the standards which are needed for the working curves and plot the concentrations of the standards against absorbance. If necessary on some instruments, convert percent absorption to absorbance. (The calibration curves are nearly linear for most elements.) Next run the samples and read the concentration of each sample from the calibration curve. For best results run standards each time a sample or series of samples are run.

D Special procedure for barium

To overcome ionization interference in the flame, add 2 ml sodium chloride solution to 100 ml sample before aspiration. Prepare standards similarly to contain 2000 mg/l sodium.

TABLE I

Metal	Wavelength mμ	Sensitivity (mg/l per 1%)[a]
Aluminum	309.3	1.0[b]
Barium	553.6	0.2
Beryllium	234.8	0.1[c]

[a] mg/l of metal for 1% absorption in an aqueous solution.

[b] 0.05 if extracted.

[c] 0.005 if extracted.

E Special procedural steps for low concentrations of aluminum and beryllium

To 100 ml of sample add 2 ml of 8-hydroxy quinoline solution and 3 ml of buffer solution. Adjust the mixture, if necessary to a pH of 8.0 ± 0.5 and then extract with 5 ml of chloroform. Repeat the extraction with a second 5 ml of chloroform and combine the two extracts. Determine the amount of metal in the organic phase by comparison with standards which have been similarly extracted.

F Aspiration of an organic solvent into the flame

When working with organic solvents, reduce the fuel-to-air ratio because the burning of the organic solvent contributes to the fuel supply and in addition to lifting the flame off the burner may produce an undesirable luminescent flame. In setting the fuel-to-air ratio of the gas mixture at the burner, start with the settings recommended by the manufacturer for the analysis and gradually reduce the fuel flow (while the solvent is being aspirated) until the flame is similar to the flame before aspiration of the organic solvent. With the nitrous oxide burner approximately double the flow of acetylene is required over that normally used for the air-acetylene burner and the acetylene should be adjusted until the flame is a rose-red.

V CALCULATION

A For aqueous sample refer directly to the calibration curve

$$\text{mg/l metal} = (\text{mg/l in aliquot}) \times D$$

where

$$D = \text{dilution ratio} = \frac{\text{ml sample} + \text{ml water} + 1\ (\text{ml acid})}{\text{ml sample}}$$

B For extracted sample refer to the extracted standard calibration curve.

$$\text{mg/l metal} = (\text{mg/l in aliquot})$$

REFERENCES

1 Atomic Absorption Methods Manual, Vol. I. Jarrell-Ash Co., Waltham, MA, 1966.

2 Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer Corp., Norwalk, CT. 1964 with suppl. of 1966.

3 Goon, E., Petley, J.E., McMullen, W.H., and Wiberly, S.E., Fluorometric Determination of Aluminum by Use of 8-Quinolinol. Anal. Chem. 25:608, 1953.

This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.

DISSOLVED OXYGEN DETERMINATION (DO) - I

Azide Modification, Iodometric Titration

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference among an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20° C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Two factors tend to confuse the reoxygenation pattern in water.

a The transfer of oxygen in air to oxygen in water is a relatively inefficient process in terms of the mass of oxygen in air at the interface and mass of oxygen transferred across the interface into the water. Diffused air aeration commonly shows that 3 to 6% of the oxygen in air fed into the bottom of an activated sludge aerator may be transferred to the liquid. Reported ranges vary from 1 to 30% or higher depending upon factors listed earlier.

b Although the process is inefficient,it occurs very rapidly. The very low solubility of oxygen in water doesn't require transfer of a large mass of oxygen per unit volume of water to change the DO concentration from 0 to 90% of saturation. Therefore, the DO may be increased very quickly by increasing the air-water interface area for samples having low % DO saturation values. In fact, it is very difficult to add reagents to samples containing no DO without transfer of small quantities of oxygen into the sample via the small surface area exposed. It is common to observe no DO in a final clarifier supernatant approaching the discharge weirs while there may be 3 to 6 mg DO/l in the effluent channel after discharge in a thin layer over the weirs. The large oxygen deficit for samples containing low percentage saturation values increases the efficiency of transfer and decreases time and energy required to raise the DO. At about 90% DO saturation, transfer efficiency is low and time and energy requirements are much greater per unit increase in DO.

c A low DO sample may be raised to 70 to 90% DO saturation by: shaking a partially filled bottle vigorously for 15 to 30 seconds, pouring rapidly from one container to another 4 to 6 times, allowing a container about 80% filled with low DO water to stand for 2 to 3 days. Diffused air aeration often is a first consideration for polluted water. This is desirable because the dust, fumes, aerosols, and water solubles in the air are small in relation to the water contaminants. A distilled or other high quality water is likely to be dirtied by aeration. Air at ambient temperature weighs approximately 1.1 g/l and contains about 230 mg of oxygen per liter. If 50% or 115 mg of this was transferred to water the net effect would increase the DO of about 14 liters of water from 0 to 8 mg per liter. This could be accomplished in 2 to 3 days of storage or in a few seconds of mixing. With high quality water such as BOD dilution water, it is essential to reoxygenate with a minimum quantity of air because of the contamination likely by contact with dirty compressed or laboratory air.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer tothe bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

III ANALYTICAL METHOD BACKGROUND

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the FWQA Quality Control Committee for relatively clean waters.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4$ (a)

$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2$ (b)

$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$ (c)

$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2$ (d)

$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI$ (e)

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents are used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the solids to settle half way and repeat the process. Reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions **are** more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as:

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

* FWQA Method

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Precise directions are given in Section V, Laboratory Procedures.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl of H_2O_2 may raise the DO titration while H_2S, 4 SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^=$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$\begin{array}{llr} 2HNO_2 + 2\,HI \rightarrow I_2 + 4H_2O + & N_2O_2 & (a) \\ \uparrow & \downarrow & \\ 2HNO_2 \leftarrow + 1/2O_2 + H_2O + & N_2O_2 & (b) \end{array}$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of HNO_2 - N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe^{+++} may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

ACKNOWLEDGMENTS

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, Environmental Protection Agency, WQO, Cincinnati, OH 45226

DISSOLVED OXYGEN DETERMINATION - II
ELECTRONIC MEASUREMENTS

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^{=}$! The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^{o} - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

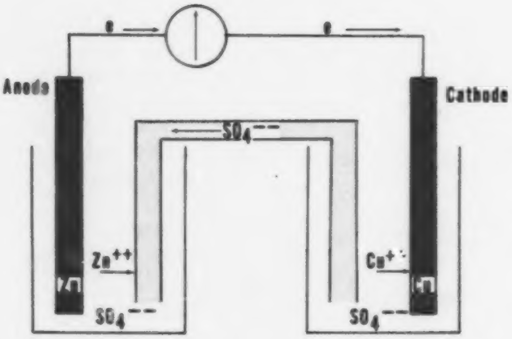
8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

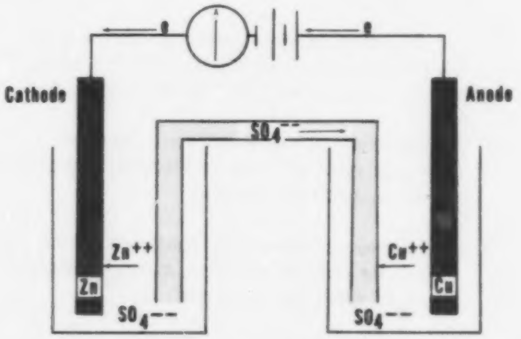
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II. A. 1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KC1 and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

a Variable line voltage or low batteries in amplifier power circuits.

b Substandard or unsteady amplifier or resistor components.

c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

d Inadequately shielded electronic components.

e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O. K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply FWQA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

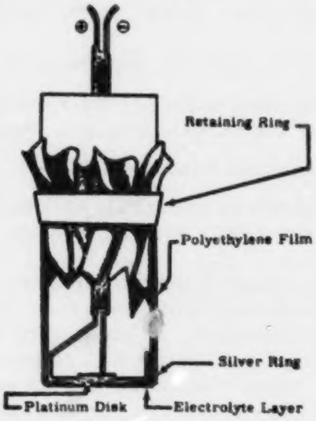

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disk
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

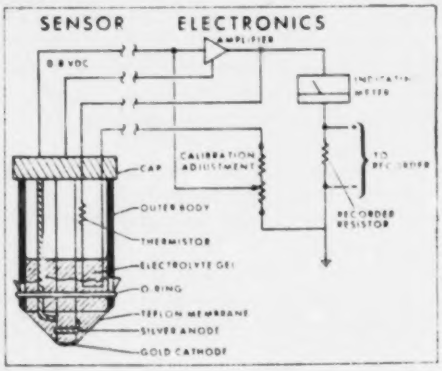

SENSOR
ELECTRONICS
AMPLIFIER
0.8 VDC
INDICATING METER
CALIBRATION ADJUSTMENT
TO RECORDER
RECORDER RESISTOR
CAP
OUTER BODY
THERMISTOR
ELECTROLYTE GEL
O RING
TEFLON MEMBRANE
SILVER ANODE
GOLD CATHODE

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

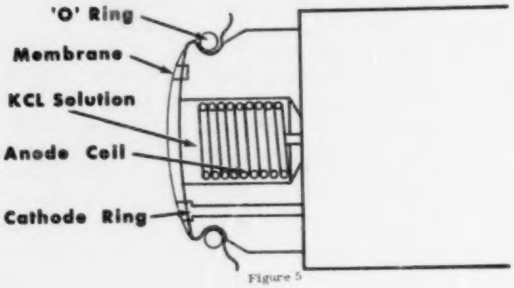
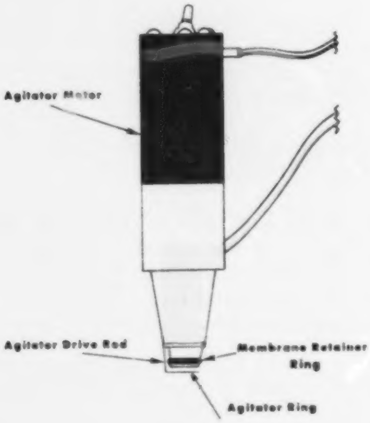

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring

Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

Model 54 Agitator Probe


Agitator Motor
Agitator Drive Rod
Membrane Retainer Ring
Agitator Ring

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

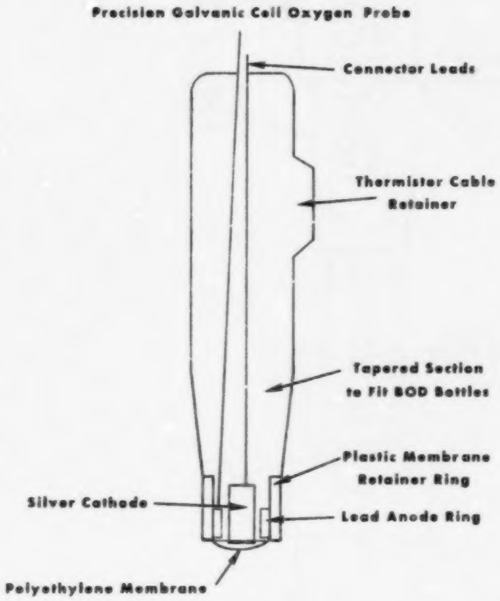

Precision Galvanic Cell Oxygen Probe
Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

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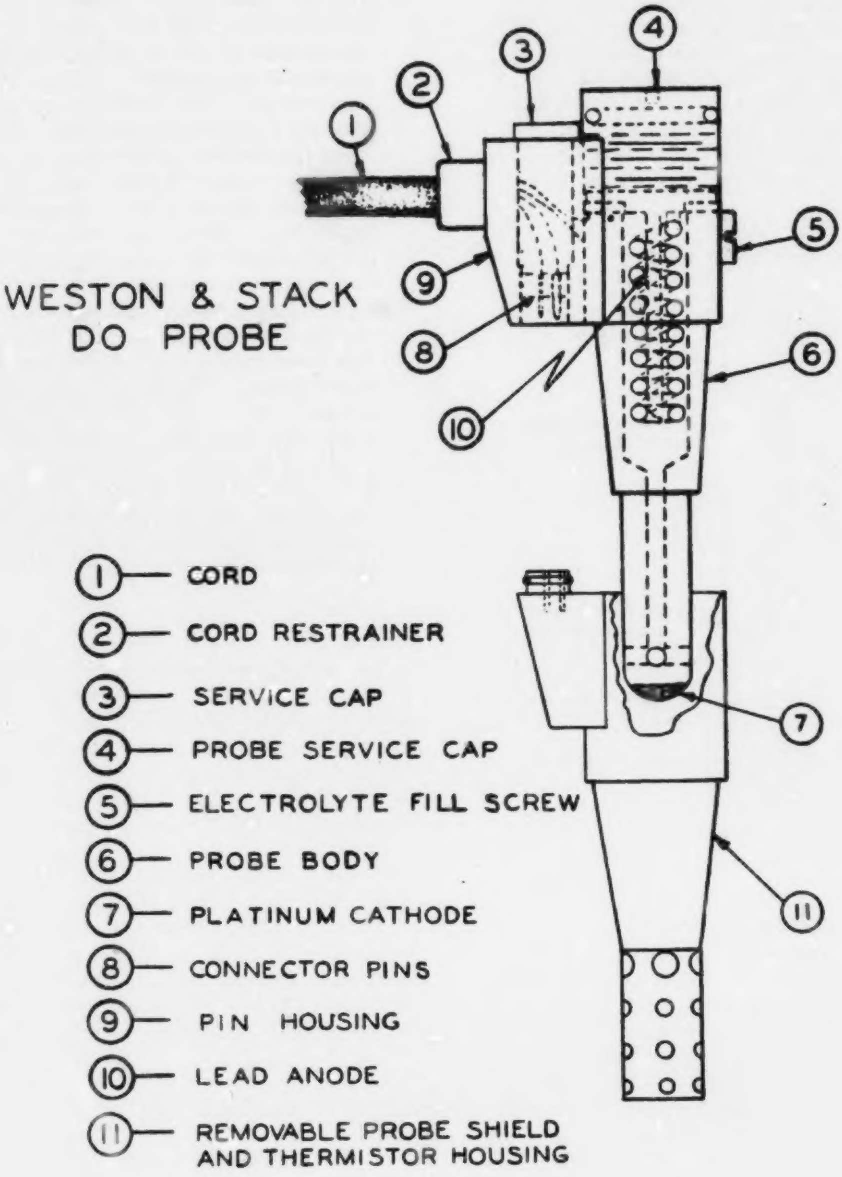
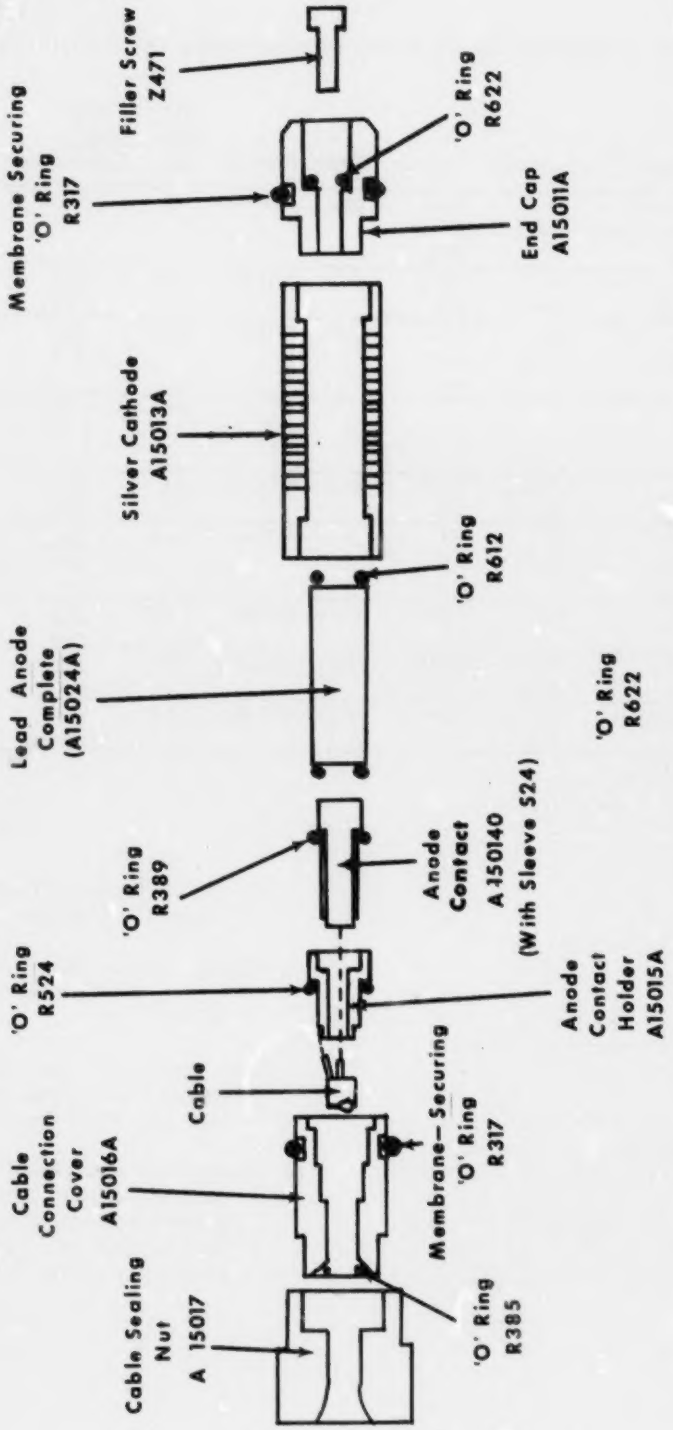

WESTON & STACK
DO PROBE
①
②
③
④
⑤
⑥
⑦
⑧
⑨
⑩
⑪
① — CORD
② — CORD RESTRAINER
③ — SERVICE CAP
④ — PROBE SERVICE CAP
⑤ — ELECTROLYTE FILL SCREW
⑥ — PROBE BODY
⑦ — PLATINUM CATHODE
⑧ — CONNECTOR PINS
⑨ — PIN HOUSING
⑩ — LEAD ANODE
⑪ — REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut A 15017
'O' Ring R385
Cable Connection Cover A15016A
Membrane-Securing 'O' Ring R317
Cable
'O' Ring R524
Anode Contact Holder A15015A
'O' Ring R389
Anode Contact A 150140 (With Sleeve S24)
Lead Anode Complete (A15024A)
'O' Ring R622
'O' Ring R612
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
End Cap A15011A
'O' Ring R622
Filler Screw Z471

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T221

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp.	Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol	polyeth	no	no		Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCL gel	pol	teflon	yes	yes	yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no*	yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes	yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv	polyeth	no	no	yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes	yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes	yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	yes	field bottle & agitator probe

*Pol - Polarographic (or amperimetric)
Galv - Galvanic (or voltametric)

REFERENCES

1 Carrit, D.E. and Kanwisher, J.W. Anal. Chem. 31:5. 1959.

2 Beckman Instrument Company. Bulletin 7015, A Dissolved Oxygen Primer, Fullerton, CA. 1962.

3 Instructions for the YSI Model 51 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

4 Instructions for the YSI Model 54 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

5 Technical Bulletin TS-68850 Precision Scientific Company, Chicago, IL 60647.

6 Mancy, K.H., Okun, D.A. and Reilley, C.N. J. Electroanal. Chem. 4:65. 1962.

7 Instruction Bulletin, Weston and Stack Model 300 Oxygen Analyzer. Roy F. Weston, West Chester, PA 19380.

8 Briggs, R. and Viney, M. Design and Performance of Temperature Compensated Electrodes for Oxygen Measurements. Jour. of Sci. Instruments 41:78-83. 1964.

9 Eden, R.E. BOD Determinations Using a Dissolved Oxygen Meter. Water Pollution Control. pp. 537-539. 1967.

10 Skoog, D.A. and West, D.M. Fundamentals of Analytical Chemistry. Holt, Rinehart & Winston, Inc. 1966.

11 FWPCA Methods for Chemical Analysis of Water and Wastes. FWPCA Div. of Water Quality Research Analytical Quality Control Laboratory, Cincinnati, OH. p. 65-68. November 1969.

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PRINCIPLES OF EMISSION SPECTROSCOPY

I INTRODUCTION

The term "spectroscopy", in its broadest sense, refers to the study of the radiations of the electromagnetic spectrum. As no single instrument exists which will separate radiation from all parts of the spectrum, it is divided into regions which are related to the different types of instruments capable of producing or measuring waves of various lengths. A diagram of the spectral distribution of energy is shown in Figure 1.

Emission spectroscopy is concerned with that region wherein radiation can be sorted out into a spectrum by means of prisms or gratings, and includes the near infrared, the visible and the ultraviolet.

Emission spectroscopy has become an indispensable part of the modern chemist's analytical methods. With it he can analyze a wide variety of substances, both qualitatively and quantitatively, for trace elements regardless of valence states.

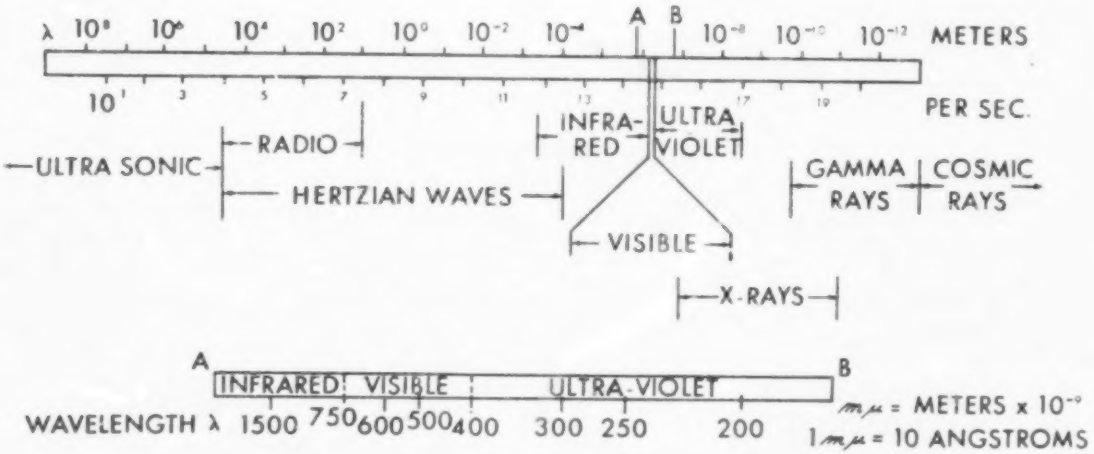
II THEORY

A Origin of Spectra - Excited atoms and atomic ions emit light of definite wavelengths. The excitation of multiple elements in a sample results in the simultaneous production of the spectra of all of these elements.

During excitation by a thermal or electrical source, the outer orbital electrons of an element absorb energy and rise to higher energy levels. These electrons then return spontaneously to their normal or ground state by a single jump or by a series of jumps. The energy emitted with each jump produces a spectral line of characteristic wave length and frequency for the particular chemical element. The combination of lines produced by the excited atoms of the element thus provides the emission spectrum of that element.

FIGURE 1

SPECTRAL DISTRIBUTION OF ENERGY



λ 10^{8} 10^{6} 10^{4} 10^{2} 10^{0} 10^{-2} 10^{-4} A B 10^{-8} 10^{-10} 10^{-12} METERS
10^{1} 3 5 7 9 11 13 17 19 PER SEC.
ULTRA SONIC
RADIO
HERTZIAN WAVES
INFRA-RED
ULTRA VIOLET
VISIBLE
X-RAYS
GAMMA RAYS
COSMIC RAYS
A
B
INFRARED
VISIBLE
ULTRA-VIOLET
WAVELENGTH λ 1500 750 600 500 400 300 250 200
$m\mu$ = METERS x 10^{-9}
$1 m\mu$ = 10 ANGSTROMS

REGION INCLUDED IN EMISSION SPECTROSCOPY

B **Types of Spectra** - There are three types of emission spectra.

1 The line spectra produced by highly excited atoms or atomic ions.

2 Band spectra which originate with highly excited molecules.

3 Continuous spectra which result when light is emitted by incandescent solids.

III INSTRUMENTATION

A The emission spectrograph consists of essentially four distinct functional parts: the excitation source, the slit, the optical system, and the recording system. A diagram of a Littrow type spectrograph is shown in Figure 2.

1 The production of line spectra for the detection or the determination of the elemental constituents in a sample requires an excitation source, such as an arc, a spark, or a flame.

2 The slit of a spectrograph permits only a narrow beam of light of mixed wave lengths to enter the instrument.

3 The optical system consists of a series of lenses and either a prism or grating to separate light rays of different wave lengths into the spectrum.

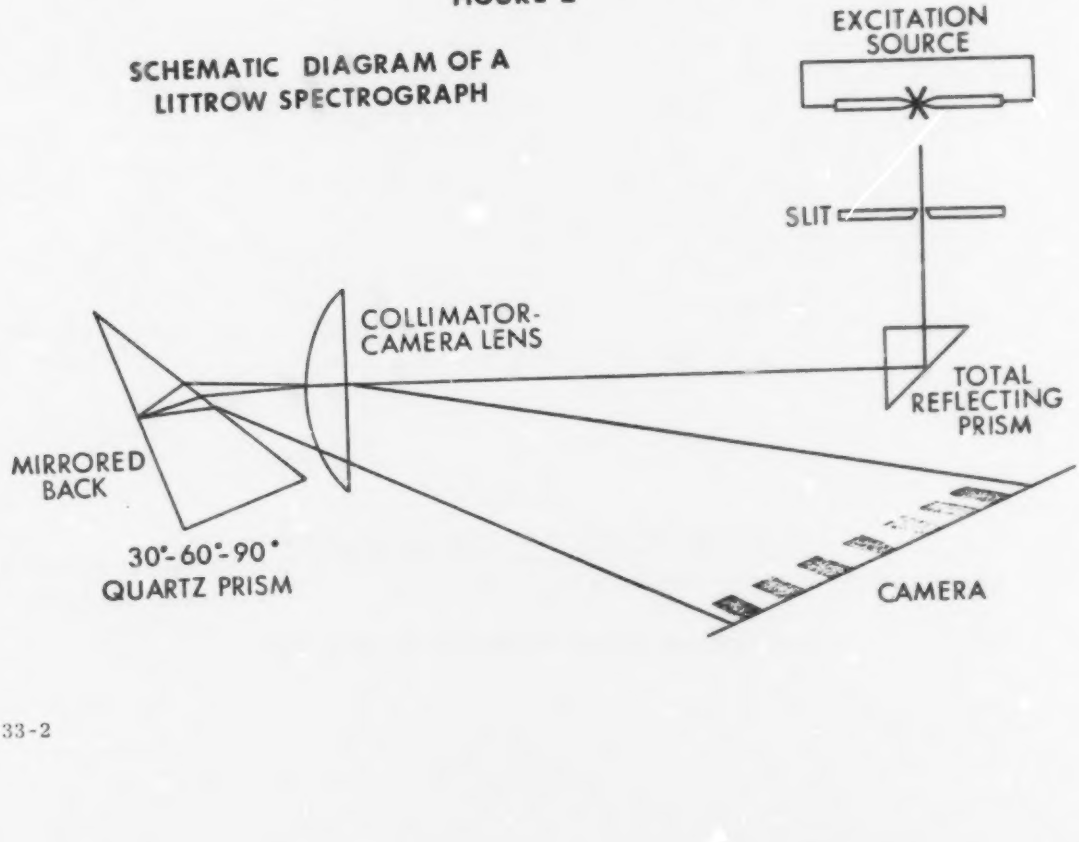
4 The recording system can be

a An eye piece such as used in a spectroscope.

b A photographic plate such as used in a spectrograph.

FIGURE 2

SCHEMATIC DIAGRAM OF A LITTROW SPECTROGRAPH



c A photoelectric cell like that used in direct reading spectrographs.

IV SELECTION OF EQUIPMENT AND COST CONSIDERATIONS

A A decision concerning the advisability of installing a laboratory for spectrochemical analysis, whether in an industrial plant, research laboratory or health department, might rest upon four main considerations.

1 Whether the analytical problems encountered have a satisfactory solution other than spectroscopy.

2 The time urgency of the work.

3 The expected volume and diversity.

4 The cost of the initial investment, together with operating expenses.

V PROBLEMS AND TECHNIQUES ASSOCIATED WITH EMISSION SPECTROSCOPY

A After the equipment has been set up properly and is ready for operating, there are many parameters to be determined before the first samples are analyzed.

1 Source - arc or spark

There are several types of excitation sources available, as shown in Table 1. Generally, the type of sample and the precision and accuracy required for the analysis will govern which excitation method will be employed.

2 Electrode

The spectrographic electrode plays an important part in the analysis. Some important features are density, shape and purity.

Table 1. EXCITATION SOURCES

TYPE	PRECISION	SENSITIVITY	RANGE	USE
D.C. ARC	Fair	Excellent	0.00001 - 1.0%	Basic sources for general qualitative analysis - soils, ores, oxides, slags, ashes, etc. Highest sensitivity of detection for trace elements.
HIGH VOLTAGE A.C. SPARK	Excellent	Fair	0.01 - 30.0%	Most stable. Use for alloying constituents in metals. Solution techniques.
LOW VOLTAGE A.C. ARC	Good	Good	0.001 - 1.0%	Quantitative determination of residual impurities for low alloying constituents in metals.
SPARK IGNITED UNI - ARC	Good	Good	0.001 - 1.0%	Combines precision of the spark and the sensitivity of the D.C. arc.
HIGH VOLTAGE A.C. ARC	Fair	Excellent	0.00001 - 1.0%	Steadier than D.C. arc - Conductors and nonrefractory materials.

a **Density** - The current and temperature obtainable are dependent on the density of the graphite.

b **Shape** - The nature of the sample being analyzed will usually determine the shape and type of electrode.

c **Purity** - Trace analysis requires that the electrodes be of very high purity.

3 Exposure conditions

Current, voltage, exposure time, etc., play an important part.

4 Photographic plates

The photographic emulsion most generally used for quantitative analysis is the S.A.#1. This emulsion responds to the spectral range from 2400 - 4400 A. It has high contrast, low background density, and low granularity.

The S.A.#2 emulsion is also desirable for trace analysis when lower contrast, higher speed, and wide latitude are needed. S.A.#2 also covers the range from 2400 - 4400A. Other photographic emulsions covering the wavelength range from 2400 - 12,000 A are available, each having specific features. The processing of these photographic plates is extremely important and the directions of the manufacturer should be followed closely.

B Qaulitative Analysis

In spectroscopy, qualitative analysis is a relatively simple process, although it can be more time consuming than a quantitative determination, especially if a complete qualitative analysis is required. The presence or absence of more than sixty of the chemical elements can be determined readily by a simple inspection of the resulting pattern of spectral lines.

All elements give specific lines when sufficiently excited, which is the basis of qualitative spectrochemical analysis.

C Quantitative Analysis

Quantitative spectrochemical analysis is based on the fact that the amount of light emitted by an element present at very low concentration is directly proportional to the number of its excited atoms present, if all other factors are kept constant. The intensity of the spectral line of the analysis element (or degree of blackening of the photographed image of the line) in an unknown sample is compared to the intensity of the corresponding line in a standard sample to provide an estimate of the concentration of the element producing that line.

D Preparation of Standards

1 Since the major constituents of a sample are important factors affecting burning qualities of the arc and intensity of resulting spectral lines, it is important that the composition of the standards approximate the samples as closely as possible. This is accomplished by preparing a synthetic matrix to approximate the average composition of the samples minus the analysis element or elements.

2 Increasing concentrations of the analysis elements are added to the synthetic matrix and are sparked under the identical conditions used for the unknown samples for calibration purposes.

3 It should be emphasized that in quantitative spectrographic analysis, the best possible standards must always be prepared. A good spectrographer is only as good as his analyses, and his analyses are only as good as his standards. Spectrographic standard solutions are prepared from Reagent Grade chemicals. Oxides are prepared where possible; however, nitrates or chlorides will usually suffice.

Occasionally, it is necessary to use special spectroscopically pure grades of certain metals. Nitric and hydrochloric acids should be redistilled; double distilled water is also suggested where possible. Borosilicate glassware, cleaned with both chromic and nitric acids, is used throughout.

4 Internal standards

In quantitative analysis, the errors caused by temperature fluctuations and wandering of the are are minimized by the use of the internal standard technique. This consists of comparing the intensity of a suitable line of analysis element in a standard and in an unknown sample to a certain selected line of another element whose concentration is fixed in all samples.

Since both the unknown and the internal standards are parts of the sampe sample, variations in time exposure, plate characteristics, and developing conditions will not affect the relative density of the two lines which are equal in intensity in the light source.

Working curves are established by plotting the intensity ratio of the analysis line to the internal standared line vs. the concentration of the analysis element.

E Accuracy

The average error in quantitative emission spectroscopy, using the photographic process, is generally between ±5 - 10%. In the direct reading process, however, where photomultiplier tubes are substituted for the photographic plate, an average error of ±2% is possible. This accuracy is more than satisfactory, considering that determinations of trace elements are being made in the parts per billion range.

VI APPLICATION OF EMISSION SPECTROSCOPY TO WATER ANALYSES

A Trace elements in water originate from a variety of sources, but can be classified into three principal groups.

1 Elements contributed by soluble materials chemically weathered from rocks and soil.

2 Elements that are selectively concentrated by vegetation and find their way to surface waters following decay and run-off.

3 Industrial sources, especially those devoted to mining, alloying, and cleaning and plating of metals.

Such sources may contribute significant quantities of trace elements to surface waters in areas both populated and unpopulated. The detection and measurement of these trace elements are difficult with conventional analytical procedures because they are not adapted to large numbers of samples, and in some cases they are not sensitive enough. The use of spectrographic procedures for routine monitoring of raw waters, however, is admirably suited to the purpose since a large number of elements may be determined simultaneously with a high degree of accuracy.

B Trace elements, whether in finished or raw waters, are generally present in concentrations too low to measure directly with the spectrograph. Therefore, a means of concentration is necessary before the examination can be completed. This can be accomplished in several ways (e. g., evaporation, precipitation, ion exchange, etc.)

1 The method employed by the Federal Water Pollution Control Administration, Water Quality Surveillance Program, consists of evaporation a filtered volume of sample at low heat on a hot plate.

A volume of sample is chosen containing 100 mg of dissolved solids and evaporated to a volume of 5.0 ml or 20,000 mg of solids/l. (The dissolved solids consist for the most part of salts of sodium, potassium, calcium and magnesium; these are the materials for which corrections must be made later.) All samples, therefore, had the same final salt concentration, but the procedure produces varying levels of sensitivity for different sources because of the dissimilar initial volumes. Thus, the limit of detection for trace elements in the Columbia River is lower than for the Missouri River because the low dissolved solids in Columbia River waters permit a larger volume of sample to be evaporated.

a This might be explained best by examining the detection limits shown in Table 2. For convenience, we have arbitrarily chosen 400 mg/l as an average TDS content of surface water, and the detection limits as shown are based on this figure. For waters of only 200 mg TDS/l, the limit would be halved; conversely, if the TDS is 800 mg/l, the limit is doubled.

Table 2. DETECTION LIMITS*, µg/l

Zinc	20	Copper	10
Cadmium	20	Silver	2
Arsenic	100	Nickel	20
Boron	10	Cobalt	20
Iron	10	Lead	40
Molybdenum	40	Chromium	10
Manganese	10	Vanadium	40
Aluminum	40	Barium	2
Strontium	4	Beryllium	0.1

*In water having 400 mg TDS/l.

b The term "detection limit" is used to indicate the lowest concentration of the material that can be distinguished from background noise or interference. The limits, as shown in the table, are based on practical observations and are not mathematically contrived figures based on signal to noise ratios or other formulas.

c When an element has not been positively identified in a sample, the detection limit, preceded by a "less than" sign (<), is used to report the analysis. It should be emphasized that the failure to detect a particular element does not mean that the element is absent; it may be present in the original sample but in a concentration below the stated detection limit. The complete spectrographic procedure has been published previously. Table 3 lists the results from analyses of over 1500 samples. The frequency of detection, the range, and the mean concentrations are given.

2 Accuracy

Recoveries were run with a composite of samples from the Missouri River. The composite was analyzed to determine what trace elements, if any, were present and at what concentrations. To a series of aliquots of this composite were added increasing concentrations of the analysis elements. The samples were acidified, evaporated on a hot plate, transferred to cylinders, and finally sparked under identical conditions. Scaler counts were converted to concentrations by using the data obtained from the synthetic matrix and the percent recoveries were calculated. A statistical treatment of this data showed a range of 80% to 113% at the 90% confidence level.

TABLE 3. SUMMARY OF TRACE ELEMENTS IN WATERS OF THE UNITED STATES*

ELEMENT	No. of Pos. Occurrences	Frequency Of Detection, %	Observed Values, µg/l		
			Min.	Max.	Mean
Zinc	1207	76.5	2	1183	64
Cadmium	40	2.5	1	120	9.5
Arsenic	87	5.5	5	336	64
Boron	1546	98.0	1	5000	101
Phosphorus	747	47.4	2	5040	120
Iron	1192	75.6	1	4600	52
Molybdenum	516	32.7	2	1500	68
Manganese	810	51.4	0.3	3230	58
Aluminum**	456	31.2	1	2760	74
Beryllium	85	5.4	0.01	1.22	0.19
Copper	1173	74.4	1	280	15
Silver	104	6.6	0.1	38	2.6
Nickel	256	16.2	1	130	19
Cobalt	44	2.8	1	48	17
Lead	305	19.3	2	140	23
Chromium	386	24.5	1	112	9.7
Vanadium	54	3.4	2	300	40
Barium	1568	99.4	2	340	43
Strontium	1571	99.6	3	5000	217

* 1577 Samples (October 1, 1962 - September 30, 1967)

** 1464 Aluminum Samples

TABLE 4. RECOVERIES OF TRACE ELEMENTS ADDED TO RIVER WATER

Element	Percent Recovery		
	Low	High	Average
Zn	86.	107.	94.1
Cd	100.	106.	103.5
As	80.	100.	93.5
B	82.	97.	88.9
P	70.	110.	91.8
Fe	85.	108.	94.1
Mo	75.	100.	83.7
Mn	94.	105.	99.1
Al	96.	125.	106.7
Be	101.	117.	109.2
Cu	96.	113.	100.8
Ag[a]			
Ni	90.	118.	103.0
Co	95.	103.	99.3
Pb	60.	93.	83.6
Cr	60.	105.	86.3
V[a]	100.	110.	104.0
Ba	70.	97.	81.7
Sr	81.	112.	95.1

a Silver precipitated and was not included.

Table 4 lists the range of these per cent recoveries along with the average recovery for each element.

C Analytical Reference Service Results

A sample containing 8 trace metals at known concentrations was analyzed by 66 participating agencies using a number of analytical methods. The mean results of all 66 laboratories, in addition to those laboratories employing spectrographic procedures, are shown in Table 5. It is apparent from this summary that the spectrograph can be used to splendid advantage on water samples.

SUMMARY

The theory and instrumentation of emission spectroscopy are presented. Consideration is given to the problems and techniques associated with trace element analysis in water using the Emission Spectrograph.

For the present time, atomic absorption is the method of choice for metals analyses in the FWQA Methods manual. (8)

When broad spectrum analyses are required, the Emission Spectrograph may be used with comparable accuracy and precision.

Table 5. SUMMARY OF RESULTS OBTAINED BY SPECTROGRAPHIC PROCEDURES ON ARS SAMPLE: WATER METALS, NO. 2.

Element	Amount added mg/1	Mean of 66 Labs	AMOUNT RECOVERED mg/1		
			Lab #71126	NWQN Lab	Lab #1615
Al	1.80	2.21	1.80	2.30	3.10
Cr	0.18	0.14	0.18	0.13	0.17
Cu	0.42	0.43	0.29	0.38	0.41
Fe	0.62 **	0.44	0.46	0.40	0.75
Mn	0.25	0.28	0.25	0.23	0.38
Cd	0.24	0.26	0.25 *	0.30	0.50
Zn	0.90	0.94	0.68	0.94	0.88
Pb	0.18	0.20	0.27	0.19	--

** Iron value was adjusted to mean value as suggested on Page 47 of ARS report.

* This result was erroneously included under Lab #7112A in the ARS report.

REFERENCES

Emission Spectroscopy

1 Machtrieb, Norman H. Principles and Practice of Spectrochemical Analysis. McGraw-Hill Book Company, Inc. 1950.

2 Clark, George L. The Encyclopedia of Spectroscopy. Reinhold Publishing Corp. 1960.

Water

1 Kroner, Robert C. and Kopp, John F. Trace Elements in Six Water Systems of the United States. J.A.W.W.A. 57:150. 1965.

2 Kopp, John F. and Kroner, Robert C. A Direct Reading Spectrochemical Procedure for the Measurement of Nineteen Minor Elements in Natural Water. Applied Spectroscopy 19:155. 1965.

3 Kopp, John F. and Kroner, Robert C. Tracing Water Pollution with an Emission Spectrograph. Journal Water Pollution Control 39:1659. 1967.

4 Haffty, Joseph. Residue Method for Common Minor Elements. U.S. Dept. of Interior Geological Survey Water Supply Paper No. 1540A. 1960.

5 Silvey, W.D. and Breman, R. Concentration Method for Spectrochemical Determination of Seventeen Minor Elements in Natural Water. Anal. Chem. 34: 784. 1962.

6 Skougstad, M.W. and Horr, C.A. Occurrence and Distribution of Strontium in Natural Water. U.S. Geological Survey Water Supply Paper No. 1396 C. 1963.

7 Clarke, F.W. The Composition of the River and Lake Waters of the United States. U.S. Geological Survey Papers No. 135. 1924.

8 FWQA Methods for Chemical Analysis of Water & Wastes, 1970.

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APPLICATION OF EMMISSION SPECTROSCOPY IN WATER ANALYSIS

I DISCUSSION OF LABORATORY RESULTS

II ANALYTICAL PROCEDURES

- A Sample Preparation
- B Exposure Conditions
- C Standard Curves
- D Photographic Vs. Direct-readers
- E Detection Limits

III CHEMICAL SEPARATIONS

- A Ion Exchange
- B Precipitation
- C Chelation and Extraction

IV INTERFERENCES

- A Effect of Major Constituents
- B Spectral Interference
- C Internal Standards

V EMISSION SPECTROSCOPY VS ABSORPTION SPECTROSCOPY

- A Arc - Spark Vs. Flame
- B Flame Vs. Absorption

VI FUTURE OF EMISSION SPECTROSCOPY

- A Plasma Arcs
- B Use of Computers

This outline was prepared by J. F. Kopp, Chief, Metals Analyses Unit, Analytical Quality Control Laboratory, FWQA.

APPLICATIONS OF INTEGRATED MULTIPARAMETER WATER QUALITY DATA ACQUISITION SYSTEMS IN POLLUTION SURVEILLANCE

I BASIC OBJECTIVES OF POLLUTION SURVEILLANCE

The fundamental objective in pollution surveillance is to accumulate sufficient knowledge of stream quality and employ this information to maximize the quality within the framework of established standards and organizations.

A Base-line Data

The quality of a particular stream must be established to determine its relationship with the overall quality imposed upon a particular basin. This is determined by:

1 Grab sample

2 Automatic water quality data acquisition.

B Data Accrued During Changes in Pollution Causes

Automatic surveillance provides transitory data during changes in pollution abatement at the source:

1 Acid mine drainage

2 Dredging operation

3 Improvements in waste treatment.

C Continuous Monitoring

Continuous surveillance of streams and rivers provides research and enforcement data and establishes the effectiveness of the pollution control program.

D Stream Management

Strategically located water quality instrumentation systems with appropriate communication linkages can be employed in the control of reservoir discharge (low flow augmentation) or to alert users (water processing plants) of pulses of low quality through the application of closed loop seromechanism principles.

II EXAMPLES OF AUTOMATIC WATER QUALITY DATA ACQUISITION SYSTEMS FOR POLLUTION SURVEILLANCE

Integrated multiparameter systems have been employed for continuous monitoring in pollution surveillance at many locations throughout the United States.

A Acid Mine Drainage

Multiparameter systems measuring

pH
Conductivity
Dissolved Oxygen
Oxidation-Reduction Potential
Temperature

were employed at Roaring Creek and Grassy Run streams for monitoring drainage from strip mines near Elkins, West Virginia.

B Buffalo River

1 An integrated system capable of measuring

pH
Conductivity
Dissolved Oxygen
Temperature

is planed for installation on the Buffalo River at the Ohio Avenue Bridge, Buffalo, New York

2 Buffalo Stream Improvement Corporation

Five companies had formulated this corporation for the primary purpose of employing Lake Erie waters for process applications. These industries are all located along the Buffalo River.

C Calumet Area Post Action Surveillance Program, FWPCA

Integrated systems have been installed by this project for the measurement of

pH
Conductivity
Dissolved Oxygen
Temperature

1 Indiana Harbor

2 Calumet River

D Detroit River

An automatic water quality monitoring system has been installed near Grosse Ile on the western shore of the Detroit River for measuring

Conductivity
Dissolved Oxygen
Temperature

E John H. Kerr Dam and Reservoir

An integrated two-parameter system has been installed at this dam on the Roanoke River for the measurement of Dissolved Oxygen and Temperature. This system measures the discharge of the dam through the application of remote sensors which are mounted at the end of a dock. The system provides both analog and digital output.

F Potomac River

The Potomac River Pollution Surveillance System is comprised of four water quality instrumentation systems integrated into a network having both 8-channel paper tape and typewritten output centrally located at the FWPCA headquarters.

1 Stations

a Arlington Bridge

Single-level sampling is employed for the following measurements:

pH
Conductivity
Dissolved Chlorides
Dissolved Oxygen
Temperature
Solar Radiation

b Wilson Memorial Bridge

Single-level sampling is employed for the measurement of:

Dissolved Chlorides
Dissolved Oxygen
Temperature

c Fort Washington and Indian Head

Two-level sampling is employed at these two stations for the measurement of

Dissolved Chlorides
Dissolved Oxygen
Temperature

Those stations which employ two-level sampling include independent parametric systems and independent intake systems for each level. The first sample is drawn approximately 10 feet from the surface, and the second level is taken approximately 30 feet below the surface.

Slide 21

2 Telemetry

The telemetry system employs Schecule 1 transmission lines and carries 62.5 milliamperes of current which is interrupted on a frequency basis in proportion to the amplitude of each parametric measurement. An interruption of 15 pulses per second represents full-scale deflection, and an interruption of 5 pulses per second represents an output of zero for any given measurement. The transmission line service is provided through the efforts of AT&T and the Chesapeake and Potomac Telephone System.

3 Central Station

The central station for the Potomac River Pollution Surveillance System is comprised of several functions:

- Digital clock
- Programmer
- Receiver
- Frequency-to-millivolt converter
- Analog-to-digital converter
- Output mechanism including 8-channel paper tape and IBM Selectric Typewriter

The operation of the system is based upon hard-wire programming, but its flexibility permits change in output format and sequence of station address.

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2 Buffalo River, Herbert H. Anderson, IJC.

3 Calumet Area Post Action Surveillance Program, Robert Bowden

4 Detroit River, Lawrence O'Leary

5 Potomac River Pollution Surveillance System, Chesapeak Bay-Susquehanna River Basins Project, Middle Atlantic Region, and Division of Pollution Surveillance

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This outline was prepared by A.F. Mentink, Chief, Instrumentation Development, Analytical Quality Control Laboratory, Division of Research, WQO

IV SPECIAL TOPICS

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EXTRACTION p-VALUES OF PESTICIDES

I INTRODUCTION

A The extraction p-value introduced by Beroza and Bowman in 1965 is based on the partition of a compound between two immiscible phases.

B The principle of partition is well known and the p-value determination is a good practical application for the identification of pesticides.

C The p-value is defined as the fraction of a solute partitioning into an upper phase of an equal volume two-phase system.

D The p-values have been determined for 131 pesticides in six binary solvent systems.

II GENERAL PROCEDURE

A A 5-ml aliquot of one of the upper phase solvents containing a given pesticide is analyzed quantitatively usually by E.C.G.C. To a second aliquot (5 ml) in a 10-ml graduated centrifuge tube, an equal volume of a lower phase solvent is added and the tube shaken for one minute. The upper phase is analyzed, quantitatively. The ratio of the second analysis to the first is the p-value. (The amount of pesticide in the upper layer divided by the total amount of pesticide determined in the first analysis.)

B Six solvent systems have been used and the p-values given. The solvent systems have been chosen to give the widest range of p-values.

C These systems are commonly used in pesticide analysis and often the solvents used for extraction do not have to be altered for p-value determination.

III NOTES ON THE PROCEDURE

The solvent systems used by Bowman and Beroza were selected as the best all around systems by a process of elimination from a larger original number of systems. The systems finally decided on are listed here:

Hexane/Acetonitrile

Hexane/90% Dimethylsulfoxide

Isooctane/85% Dimethylformamide

Isooctane/Dimethylformamide

Heptane/90% Ethanol

Isooctane/80% Acetone

IV ADVANTAGES

A The p-value determinations are practically independent of concentration and the presence of extract interferences.

B The sensitivity is in the nanogram range.

C The method adds another confirmation of identity by measuring another physical property.

D It is simple and quick giving results in a matter of minutes.

E The procedure can be used for mixtures of insecticides.

F No special equipment is required.

G Pesticides having the same retention time on a G.C. column can be quickly distinguished using p-values and in many cases valuable time saved by eliminating the need for G.C. column changes.

H Other solvent systems may be substituted for the six originally used.

I If an unknown compound is being analyzed, it is not necessary to know the exact amount, only the relative amounts.

V DISADVANTAGES

Some compounds are reactive in a specific solvent system or react when injected into the G. C. unit and these p-values are not reliable.

VI APPLICATIONS

The following table shows a practical application on an actual sample of fish blood:

p-VALUES

Sample	Hexane / 80% Acetone	Isooctane / 80% Acetone	Hexane / Acetonitrile
Blood Extract	0.95	0.85	0.26
Aldrin	0.96	0.98	0.73
Parathion	0.75	0.76	0.044
Malathion	0.52	0.46	0.042

Aldrin, parathion, and malathion all had identical retention times on the G. C. column used. The gas chromatogram of the sample showed a peak at the same retention time, but with p-values was shown to be none of the three insecticides.

REFERENCES

1 Beroza, M. and Bowman, M.C. Identification of Pesticides at Nanogram Level by Extraction p-Values. Anal. Chem. 37, 291. February 1965.

2 Bowman, M.C. and Beroza, M. Extraction p-Values of Pesticides in Related Compounds in Six Binary Solvent Systems. Journal of the A.O.A.C. 48, 5, 943. 1965.

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INSTRUMENTAL ANALYSIS OF PHENOLS AND MERCAPTANS BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

I INTRODUCTION

A Foreword

To aid in monitoring waterway, sensitive, rapid and more specific methods are needed for measuring traces of mercaptans and phenols that adversely affect the palatability of water. In addition, certain types of phenols, e.g., dinitrophenol, pentachlorophenol, have certain tolerance limits suggested because of their toxicity. Because of the multitude and complexity of our manufacturing processes, one can assume that the measurement for specific compounds will be more difficult to conduct in order to establish responsibility for violations of water quality standards and to secure abatement of pollution.

This procedure is developed for the qualitative and quantitative weak acid analyses of grab samples at sensitivities 500 to 1000 times greater than present methods. In addition, this method eliminates the tedious and time-consuming cleanup procedures that are characterized by inherent losses and decomposition.

B The entire procedure for the analysis involves collection, extraction, derivative preparation and gas chromatographic analyses.

C Equipment

1 All glassware are cleaned in a detergent water solution, rinsed well with distilled water, and then heated at 1000°F in a mechanical convection oven for thirty minutes. After cooling, all orifices of the clean glassware are wrapped and sealed with fresh aluminum foil.

2 Extractions may be carried out with a liter separatory funnel or a semiautomatic extractor using 15% methylene chloride in hexane. (Certified D-37 Methylene chloride may be obtained from Fisher Scientific Company. Purified hexane, distilled in glass, is purchased from Burdick and Jackson Laboratories, Muskegon, Michigan.)

3 Solvents may be removed in a rotating vacuum evaporator or in an all-glass distillation system attached to the building vacuum system or to a mechanical vacuum pump. The latter system consists of a round bottom flask fitted to a simple Claisen distillation head with a thermometer well, water condenser, adaptor, distillate receivers of the "udder" type, and a dry ice trap. The system is then attached to a building vacuum line or a mechanical pump.

4 Derivatives are prepared in an all-glass equipment, consisting of a water condenser fitted with 19/38 joints and capped with a tin foil dust trap, and a round bottom flask of 100 to 250 ml capacity.

5 A Perkin-Elmer Gas Chromatograph, Model 811, with tritium foil Electron Capture Detector of parallel plate design is used. An 8 foot by 0.25 inch aluminum tubing containing a 50-50% mixture of 5% F.S. 1265 and 3% D.C. 200 supported on Chromosorb P of 60-80 mesh size is employed at 200°C. Nitrogen flow is 40 ml per minute. The sensitivity is set at an attentuation of x-50 and x-100, while the power supply is operated on DC 10 volts.

The gas chromatographic column, through previous conditioning with injections of aldrin, endrin, etc., and through several months use for pesticide analyses, yields excellent separations and is recommended for the analyses.

II PROCEDURE

A Collection

Collect the water sample in a liter sample jar. Cap the completely filled jar with a screw cap fitted with a Teflon liner. Preserve, prior to extraction, the samples in a refrigerator.

B Extraction

To a liter separatory funnel, add 65 ml of 15% dichloromethane in hexane. Of the 1 liter of water sample, take and introduce a 700 ml portion into the separatory funnel. Add three drops of concentrated hydrochloric acid so that the water sample is slightly acid. Tilt the separatory funnel back and forth for several minutes to extract the organic materials. Set the funnel with its contents on a ring stand in a well-ventilated hood. After the lower layer is drawn off, add an additional 65 ml of the 15% dichloromethane-hexane mixture. Repeat the extraction process until 200 ml of extract are collected. Wash once with 15 ml of organic-free, distilled water. Collect the lower dichloromethane-hexane layer in a 250 ml round bottom distillation flask after passage through a short-stemmed funnel fitted with fluted filter paper that is filled with several grams of anhydrous sodium sulfate. Remove the solvent with aid of a rotating vacuum flask in which the contents of the flask are heated on a water bath at 50°C to 65°C. Collect the condensate in a dry ice trap which is attached to a mechanical vacuum pump or a building vacuum line. For the more volatile mercaptans, add 2 ml of 0.86 N alcoholic KOH prior to the solvent removal step. (NOTE: An all-glass distillation equipment may be used in place of the rotating vacuum evaporator.)

C Conversion to a Derivative

Add to the dried residue 8 ml of 0.8N alcoholic KOH, 20 ml of distilled absolute ethanol, and 10 ml of acetone. Stir the mixture with the aid of a magnetic stirring bar placed in the flask mounted above the stirring motor. After 5 minutes, shut the stirrer off and add the equivalent of 20 mg of the pentafluorobenzyl bromide reagent contained in 2 ml of alcohol. (Prepared in the hood by diluting 1.00 gram of reagent to 100 ml.) Stir the mixture for five minutes. Place the flask and contents on a water bath. To the flask, attach upright a water condenser fitted with a 24/40 joint. Cover the top of the condenser with aluminum foil fitted tightly over the top of the condenser.

Permit the reaction mixture to reflux for two hours. Cool. Rinse the insides of the condenser with 10 ml of 15% dichloromethane-hexane mixture. Remove, and fit the flask containing the reaction mixture to a rotating evaporator or to an all glass vacuum distillation system in which the distillate may be collected and condensed in a dry ice condenser.

Extract the dried residue with 90 ml of 15% dichloromethane in hexane and 20 ml of water in the distillation flask. Rotate thoroughly the flask and contents to extract the material at the sides of the flask. Transfer the extract layer and the water layer to a separatory funnel. Shake gently for five minutes. Permit the two phases to separate. Filter the lower extract through anhydrous sodium sulfate (3 grams) contained in a fluted filter paper held in a short-stem glass funnel. Determine the pH of the aqueous wash (top layer) which should be alkaline. Collect the filtrate in a 250 ml round bottom flask. Wash the filter paper and Na_2SO_4 with 10 ml of 15% CH_2Cl_2 in hexane. Remove the solvents in the manner described previously. Weigh the dried residue and then dilute to 100 ml with 3% acetone in hexane. Dilute 1 ml of the initial dilution to 100 ml with 0.3% acetone in hexane. From this solution, inject several microliters of solution into the gas chromatograph.

III ANALYSES - ELECTRON CAPTURE GAS CHROMATOGRAPHY

A Identification

For the identifications of phenols, as ethers, make a 2 µl, 4 µl, and 6 µl injections at proper intervals. Repeat this procedure two more times. Determine the injection volume - response curve. Dilute a measured 80 mg of known pentafluorobenzyl phenyl ether to an equivalent volume of 100 ml with 3% acetone in hexane. Dilute 1 ml of the initial solution to 100 ml with 0.5% acetone in hexane. From this second dilution, inject a 4 µl volume or an appropriate volume into the electron capture gas chromatograph. Repeat the injection two times and calculate the average retention time. Divide the average of the retention times of each peak from the unknown by the average of the retention time of pentafluorobenzyl phenyl ether. Compare the relative retention time of each unknown ether with those listed on Table I. Check the absolute retention time of the unknown with that of the prepared known derivative for comparison. The two retention times should check.

TABLE I

PROPERTIES OF ETHERS AND THIOETHERS

Pentafluorobenzyl Aryl (or Alkylthio) Ether	M.P.°C	R.I. $n_D^{24°}$	Yield %	Retention Time (Min.)	Relative Retention (To Phenolic Ether)
(1) t-Butylthio		1.4744	99	1.61	0.67
(2) n-Butylthio		1.4676	98	1.85	0.77
(3) Phenyl	75- 76		96	2.40	1.00
(4) 2-Tolyl	78- 79		88	2.72	1.13
(5) 4-Tolyl	74- 75		85	2.91	1.21
(6) 2, 4 Xylyl	50- 52		94	3.27	1.36
(7) 2-Chlorophenyl	91- 93		99	3.74	1.56
(8) 4-Chlorophenyl	66- 67		94	4.11	1.72
(9) 2,4-Dichlorophenyl	73- 74		86	5.91	2.46
(10) n-Decylthio		1.4662	86	7.36	3.06
(11) n-Undecylthio		1.4665	100	9.68	4.03
(12) Naphthyl-2	132-133		84	10.46	4.35
(13) n-Dodecylthio		1.4668	96	12.91	5.37
(14) 2-Nitro, 4-Chlorophenyl	99- 99.5		92	16.26	6.76

B Quantitative Analyses

Make the calculations of the amount of unknown present in each identified peak by comparing the area under the known peak and the amount injected for the known peak versus the area under the identified peak and the amount injected for the unknown.

$$\frac{A}{W} = \frac{Ax}{Wx}$$

(Where A is area of curve obtained by injecting the known derivative, W is the weight injected of the known derivative (nanograms), and Ax is the area of the curve obtained by injecting the derivative of unknown origin. Wx is the weight of the derivative of unknown origin.) Calculate the weight of the identified derivative by solving for Wx:

$$Wx = \frac{W \cdot Ax}{A}$$

Then multiply Wx (nanograms) by the dilution factor, e.g. 10,000. Calculate the amount of the phenol by using the fraction formed by the molecular weight of phenolic compound divided by the molecular weight of the derivative (ether of the phenolic compound).

Thus for paracresol, use the following calculations:

$$\frac{Wx\ (\text{nanograms}) \times 10^4 \times 108.14}{0.7 \times 288.23} =$$

weight of the paracresol in the original grab sample per liter.

C Mercaptans

Determine the mercaptans as pentafluorobenzyl alkyl (aryl) thioethers in a similar manner with use of the sulphur cell microcoulometric titration gas chromatography.

D Interference

None known at present time.

REFERENCE

1 Breidenbach, A.W. et al. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. U.S. Department of the Interior, Publication WP-22. November 1966.

2 FWPCA Method for Chlorinated Hydrocarbon Pesticides in Water and Wastewater. U.S. Department of the Interior. Federal Water Pollution Control Administration, Division of Water Quality Research, Analytical Quality Control Laboratory. April 1969.

3 Kawahara, F.K. Anal. Chem. 40, 1009. 1968.

4 Kawahara, F.K. Ibid. 40, 2073. 1968.

5 Kawahara, F.K. Environmental Science and Technology. (To be published)

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*Mention of products by manufacturers is for identification only and does not imply endorsement by the Federal Water Pollution Control Administration of the U.S. Department of the Interior.

SPECIAL INSTRUMENTAL METHODS - MASS SPECTROMETRY AND NMR

I INTRODUCTION

Mass spectrometry and nuclear magnetic resonance spectrometry (NMR) are now widely used in organic analytical chemistry, but have only begun to find application to the analysis of chemical pollutants in the aquatic environment. Now that the cost, and in some cases the complexity of these instruments have decreased, they will undoubtedly become quite important in water pollution analysis during the next few years. This is especially true of mass spectrometry, and the majority of this lecture time will be devoted to examples of applications of this instrumental method to water pollution problems encountered at the Southeast Water Laboratory. Examples of NMR applications will also be given, as well as a brief discussion of the cost and manpower requirements, sample requirements, operating principles, and informational output of both methods. Inorganic mass spectrometry, a very useful method for elemental analysis of water samples, will not be discussed since the operating techniques and sample requirements are quite different from those for organic mass spectrometry.

II MASS SPECTROMETRY

A Economic factors

1 Mass spectrometers range in price from \$20, 000 to \$200, 000 or more. Ordinary general purpose instrumentation begins at between \$30, 000 and \$35, 000.

2 Mass spectrometers are complicated instruments and generally are "down" from 10% to 50% of the time, depending upon the make and complexity of the spectrometer. Maintenance requires money for parts and service.

3 Time is an important economic factor. To operate, maintain, and interpret spectra from a mass spectrometer of medium complexity with a reasonable work load, at least one trained person is required full time.

B Sample requirements

1 Solid samples can be handled by direct probe accessories. From 10^{-12} to 10^{-7} grams of sample are required, depending upon sample volatility and instrument sensitivity. Much information can be obtained from a sample of low purity, say 90%, and some useful information can be obtained from simple mixtures. Viscous liquids are also generally handled in the direct probe.

2 The liquids sample inlet on a mass spectrometer is generally used for volatile liquids. Sample size is between 0. 1 and 1 mg, and, here again, much information can be obtained from impure samples or in certain cases, samples in solution.

3 Gaseous samples are handled in special gas sampling flasks and inlets.

4 Gas chromatographic inlet system

The interfacing of a gas chromatograph to a mass spectrometer provides a very useful sample introduction mode. Using this technique, any compound that will chromatograph is eluted from the GC column, passes through a special sample enricher, and goes into the mass spectrometer proper. The enricher, known as a separator, removes the GC carrier gas preferentially, thus increasing the concentration of the eluted sample compound while lowering the pressure to a level suitable for the mass spectrometer. The application of this GC introduction mode to water pollution analysis is obvious. An organic extract of a water sample is chromatographed under previously established conditions, and a rapid scan (1 to 3 seconds) is made of each eluting component of the extract to produce the corresponding mass spectrum. Sample size required here is usually on the order of 0. 1 μg of each component, although useful spectra can sometimes be obtained with nanogram quantities.

C Operating principles

Regardless of which sample introduction mode is used, the sample must be in the gaseous state for the process of ionization. The sample molecules are bombarded by a beam of electrons of variable energy in the ion source (Figure 1) to produce positive

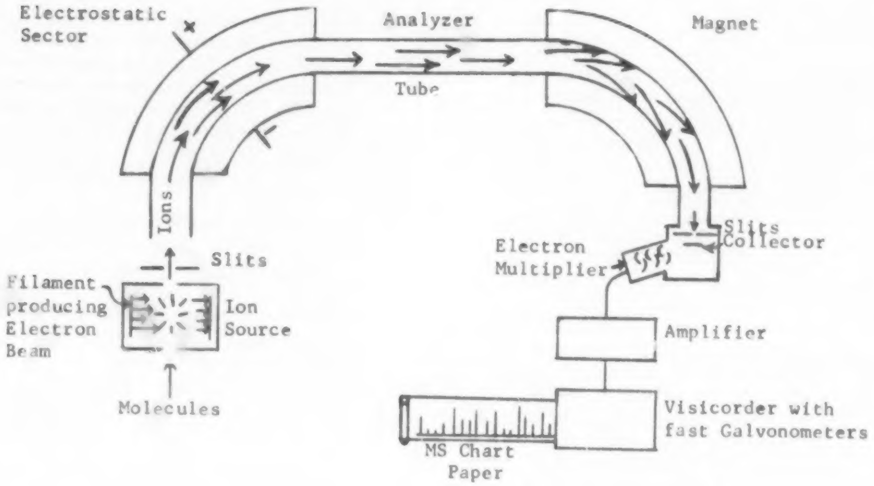

Electrostatic Sector
Analyzer
Tube
Magnet
Ions
Slits
Filament producing Electron Beam
Ion Source
Molecules
Electron Multiplier
Slits
Collector
Amplifier
Visicorder with fast Galvonometers
MS Chart Paper

Figure 1. Block diagram of a double-focusing mass spectrometer.

ions. These ions are swept through a series of slits and down the analyzer tube by a strong electrostatic field. The source and tube must be maintained at a low pressure ($<10^{-5}$ torr) to minimize collisions of the ions with each other or with air molecules. The ions pass through a magnetic field where they are deflected to different degrees based on their mass to charge ratios. By sweeping the magnetic field strength, ions of successively increasing mass are sequentially brought into focus at the collector. In a double-focusing mass spectrometer, resolution is improved by an electrostatic sector which renders the ion beam monoenergetic by velocity focusing before it arrives at the magnetic focusing sector. The electron multiplier amplifies the signal received at the collector and the resulting signal is further amplified to drive a set of galvonometers which make a trace on a photographic chart paper.

D Information output

1 All fragment ions of a molecule give rise to peaks of certain intensities with positions on the abscissa of the spectrum corresponding to ion masses (m/e values). This information constitutes the mass spectrum, and comparison with the spectra of known compounds give definitive compound identification. **Figure 2 is a plot of a typical mass spectrum, that of bis-(2-ethylhexyl) phthalate, compared with that of a compound extracted from the Tennessee River. They are practically identical.**

2 The molecular weight of a compound can often be obtained from its mass spectrum. **The fragment ion of greatest mass in most cases corresponds to the molecular weight of the compound** (Figure 2, $M^+=390$), **having been created by loss of only one electron during electron bombardment.**

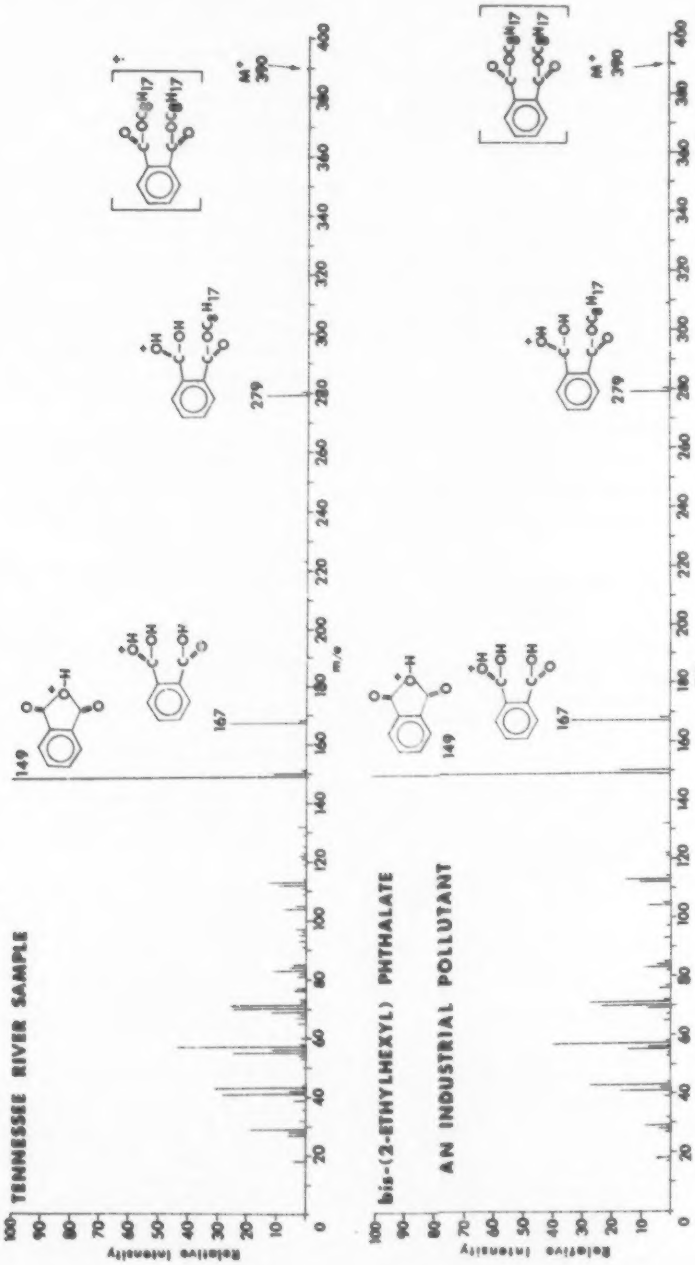

TENNESSEE RIVER SAMPLE
bis-(2-ETHYLHEXYL) PHTHALATE
AN INDUSTRIAL POLLUTANT
Relative Intensity
m/e
149
167
279
M^+ 390
OC_8H_{17}
OH
0
10
20
30
40
50
60
70
80
90
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400

Figure 2

3 The masses of important fragment ions indicate the empirical formulae of the ions and hence give clues as to structures of parts of the molecule (Figure 2).

4 Isotope peaks are helpful in determining if a molecule contains chlorine, bromine, sulfur, mercury, and other atoms which have more than one highly abundant naturally occuring isotope.

5 High resolution mass spectrometry is capable of providing almost the exact mass (to four decimal places) of the parent ion and of the important fragment ions from the molecule. This degree of accuracy of mass determination eliminates all but one or two possible empirical formulas for these ions, thus providing very important clues to the molecular structure.

III EXAMPLES OF APPLICATIONS OF MASS SPECTROMETRY TO WATER POLLUTION PROBLEMS

(Corresponding spectra will be projected and discussed.)

A The identification of S, S, S-tributyl phosphorotrithioate in Charleston Harbor, S. C., depended to a large extent on molecule weight determination by mass spectrometry.

B The identification of phenyl mercuric chloride, believed to be the cause of a fish kill in Boone Lake, Tennessee, involved the observation of mercury and chlorine isotope peaks in the mass spectrum.

C Diazinon was separated from a Tombigbee River water extract by thin layer chromatography, removed from the adsorbent, and analyzed by mass spectrometry.

D The analysis of petroleum rocket fuel residues extracted from water illustrate the applicability of mass spectrometry to oil pollution analysis.

E Bis-2-(ethylhexyl) phthalate was identified as a pollutant in the Tennessee River with the aid of mass spectrometry (Figure 2).

F An actinomycetes metabolite, responsible for earthy taste and odor problems in an Ohio municipal water supply, was identified as 2-methylisoborneol. The mass spectral fractionation pattern of the unknown compound was important in the elucidation of its structure.

G Nonylphenol has been extracted from a treated woolen mill effluent, collected upon elution from a gas chromatographic column, and identified by mass spectrometry using the direct probe accessory.

H Several terpenes and terpene derivatives in the treated effluent of a kraft paper mill have been identified by combination gas chromatography-mass spectrometry (GC-MS). The complexity of the gas chromatogram illustrates both the great problems encountered in waste characterization and the beauty of the GC-MS technique; whereby, many of the individual components can be identified.

I Three resin acids and several fatty acids have been identified in the effluent and in the foam downstream from a paper mill. The methyl esters of the acids were prepared by reaction with diazomethane, and the volatile esters were analyzed by the combined GC-MS technique.

IV NUCLEAR MAGNETIC RESONANCE SPECTROMETRY (NMR)

A Economic factors

1 NMR instrumentation ranges in price from about $20,000 for a "desk-top" low resolution (60 MHz) spectrometer to $85,000 for a 100 MHz instrument with common accessories.

2 NMR equipment is not as mechanically complex as mass spectrometry instrumentation; down time is generally only 10% to 20% and a full time operator is not necessary for a reasonable work load. However, an experienced operator is necessary for optimum output.

B Sample requirements

1 Conventional NMR samples must be in the liquid phase, either neat or in solution. Solvents must be deuterated or aprotic (e. g., CCl_4, CS_2) since any protons present absorb energy to give spectral interferences. Milligram quantities of sample (in solution) are required except that with particularly sensitive instrumentation 0.1 mg can sometimes give good spectra, and with

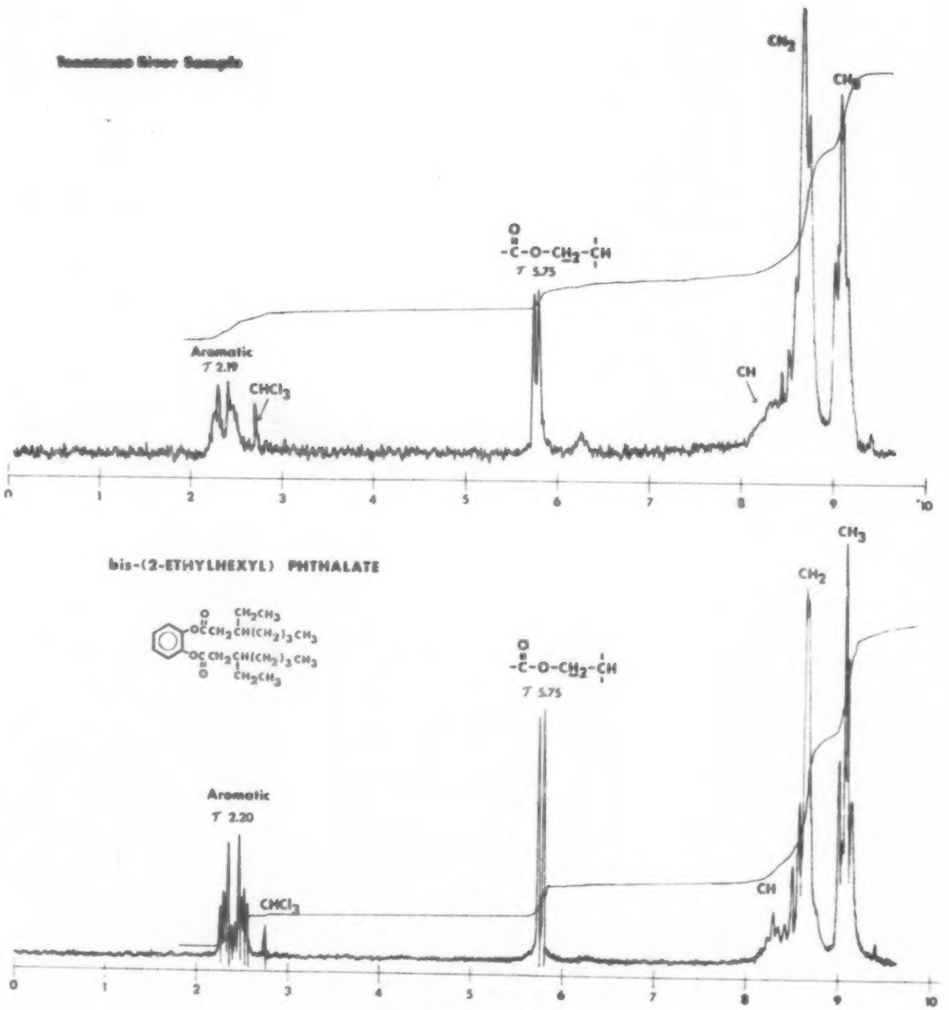

Tennessee River Sample
CH2
CH3
O
-C-O-CH2-CH
τ 5.75
Aromatic
τ 2.19
CHCl3
CH
0
1
2
3
4
5
6
7
8
9
10
bis-(2-ETHYLHEXYL) PHTHALATE
O
OCCH2CH(CH2)3CH3
CH2CH3
OCCH2CH(CH2)3CH3
O
CH2CH3
CH3
CH2
O
-C-O-CH2-CH
τ 5.75
Aromatic
τ 2.20
CHCl3
CH
0
1
2
3
4
5
6
7
8
9
10

Figure 3

time-averaging computerization to enhance the signal to noise ratio, less than 10 micrograms is sometimes sufficient. A purity of 90% is usually adequate to give useable spectra, and simple mixtures can often yield useful information.

2 A lack of sensitivity (a factor of 10^3 to 10^6 times as much sample is required as for mass spectrometry) and the longer time required for analysis are the principal disadvantages of NMR. The method is extremely valuable; however, in detailed structural elucidation, providing information as to the types and environments of hydrogen atoms which cannot be obtained by any other instrumentation.

C Information output

Hydrogen nuclei absorb energy in the radiofrequency range at certain frequencies depending on their environment. Thus, signals at certain frequency areas of the spectrum are characteristic of methyl, methylene, aldehydic, aromatic, etc., protons. (Figure 3). The NMR is capable of integrating these signal areas relative to each other; therefore, allowing calculation of the number of each type of proton in the molecule. In the case of simple mixtures, this feature often allows the calculation of percentage composition of the components.

In addition to absorbtion of radiofrequency energy, nuclei with magnetic moments, such as hydrogen, phosphorus, and carbon-13, interact with each other when in the same proximity to cause "splitting" of the proton signal peaks into a larger number of smaller peaks (Figure 3). This is known as "spin-spin coupling" and is a very informative element of the NMR method. Splitting rules allow the calculation of the number of peaks resulting from the coupling of one proton with other adjacent protons. Thus, the degree of splitting of a particular hydrogen signal is indicative of the protonic environment of the hydrogen atoms, and this allows important deductions to be made regarding the structure of the molecule.

D Examples of applications of NMR to water pollution problems

1 The NMR spectrum of a phthalate ester isolated from the Tennessee River provided the prime evidence for the detailed structure of the molecule, especially of the 2-ethylhexyl moities. It was later shown that this spectrum matched that of bis-(2-ethylhexyl) phthalate (Figure 3).

2 The NMR spectrum of the actinomycetes metabolite extracted from an Ohio municipal water supply showed the compound to have four tertiary methyl groups.

3 The frequency of the methylene absorption signals of the NMR spectrum of a toxic compound isolated from Charleston Harbor, S. C., indicated the compound to be the phosphate derivative of a phosphite ester present in an industrial effluent discharging into the harbor.

REFERENCES

1 McLafferty, F. W. Interpretation of Mass Spectra, W. A. Benjamin, Inc., New York, 1966.

2 Silverstein, R. M., and Bassler, G. C. Spectrometric Identification of Organic Compounds, John Wiley and Sons, Inc., New York, 1963.

3 Willard, H. H., Merritt, L. L., Jr., and Dean, J. A. Instrumental Methods of Analysis, Chapters 6 and 16, Fourth Edition, D. Van Nostrand Co., Inc., Princeton, N. J., 1965.

4 Teasley, J. I. Identification of a Cholinesterase - Inhibiting Compound from an Industrial Effluent, Environ. Sci. & Technol. 1(5):411, 1967.

5 Medsker, L. L., Jenkins, D., and Thomas, J. F. Odorous Compounds in Natural Waters, Environ. Sci. & Technol 3(5): 476, 1969.

This outline was prepared by Arthur W. Garrison, Research Chemist, Southeast Water Laboratory, WQO.

DOSAGE AND PROCESS CONTROL

The quality of treated water and the chemicals costs for its production are dependent on proper control of the coagulation-flocculation processes. Laboratory and in-plant control are essential to proper coagulation treatment for two basic reasons: First, plants which employ coagulation usually treat surface waters which may be subject to wide variations in quality and are much more likely to be contaminated than well supplies. Second, the best and most economical treatment of such waters cannot be predicted from known relationships but must be checked by one or more testing or monitoring techniques. The coagulation and flocculation of water is influenced by a number of interrelated factors such as pH, color, turbidity, mineral content and composition, temperature, the duration and degree of agitation during flocculation, and finally, the characteristics of the coagulant or flocculation aid in use. Unfortunately, the optimum conditions for coagulation cannot be determined from the results of a chemical and physical analysis of the water, but must be determined empirically. The type and frequency of tests depend largely on the character of the raw water and on the plant design. Where possible, laboratory investigations of treatment should begin before the plant is designed and constructed, since operation and control can be simplified if the plant is designed specifically to handle the particular raw water, especially if that water is subject to wide variations in quality. Because each water plant may require its own specialized combination of tests and methods for performing these tests, the subject of dosage and process control will be treated here only in a general way.

I THE JAR TEST

The single most widely used test to determine dosage and other parameters is the "jar test." The equipment for this test and the directions for the proper performance have been published. The jar test attempts to simulate the full-scale coagulation-flocculation process and has remained the most common control test in the laboratory ever since its introduction in 1918. Since the intent is to simulate an individual plant's conditions it is not surprising that there has been no standardization of the test. The jar test as variously performed does, however, have some elements of conformity. In its essentials, the jar test simply consists of a series of sample containers, the contents of which can be stirred by individual mechanically operated stirrers. Water to be treated is placed in the containers, and the treatment chemicals are added while the contents are being stirred. The range of conditions, for example pH and coagulant dosages are selected to bracket the anticipated optima. After a short, 1 - 5 minute period of rapid stirring to ensure complete dispersion, the stirring rate is decreased and flocculation is allowed to continue for a variable period, 10 - 30 minutes or more, depending on the simulation. The stirring is then stopped, and the floc are allowed to settle for a selected time. The supernatant is then analyzed for a variety of parameters.

The interpretation of the results of this test requires some determinative measure of the flocculation. A number of approaches have been used: (1) visual evaluation of floc size, (2) visual or photometric measurement of supernatant clarity or color, (3) time of appearance of first floc, (4) chemical determination of residual coagulant in supernatant, Figure 1, (5) rate of settling of floc, Figure 2, (6) measurement of the electrophoretic mobility (zeta potential) of the particles, and (7) more recently, the filterability characteristics either through paper, membranes or through miniaturized sand filters, Figure 3.

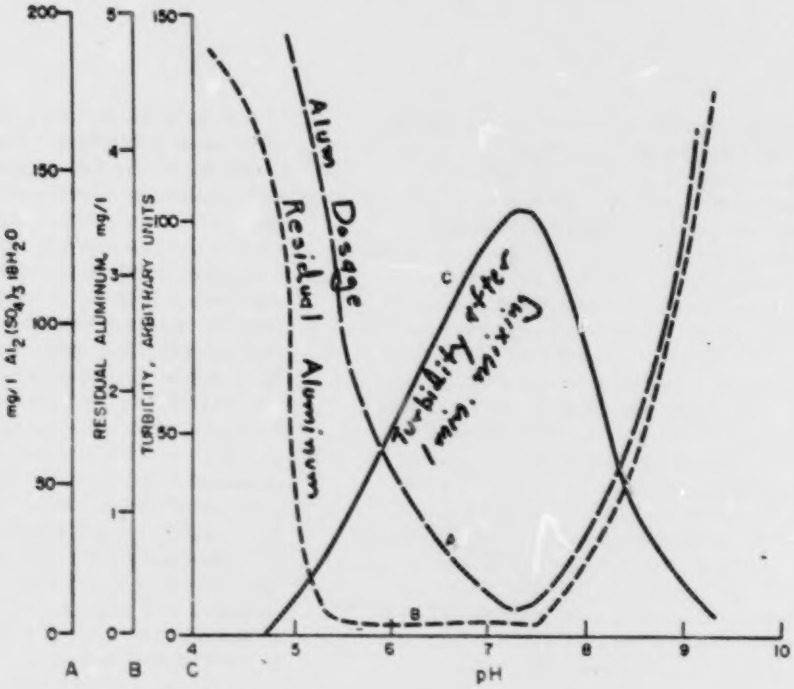
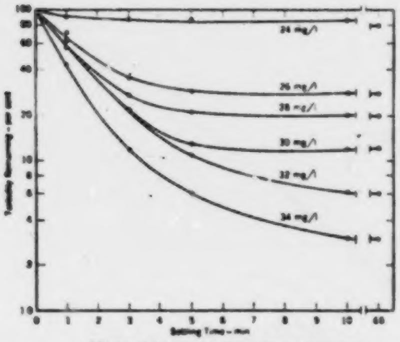


FIGURE 1
COMPARISON OF THE EFFECTS OF pH

Since the objective of coagulation-flocculation is to destabilize the particles so that removal can be obtained by sedimentation and filtration, residual turbidity measurement has been a most important parameter. Turbidity, as the word is understood in the water field, is simply an optical property of the suspended particles in the water, and it is not a direct assessment of the quantity present. Since the measured optical properties of particles vary depending on their size distribution, refractive index, concentration, angle of observation with respect to the light source and on the instrument geometry, it is not surprising that numerical values for turbidity may not be compared between plants. The problem is further complicated because of the lack of a generally accepted reference turbidity material. In the usual determination of turbidity, the light scattered by the particles in the water is compared with that scattered by a dilute clay suspension which in turn is standardized by the Jackson Candle method. The problem here is that the Jackson Candle method cannot be used for turbidities less than 25 units, and that clay suspensions are neither standard nor reproducible among laboratories. There are ongoing efforts to standardize this test particularly



Effects of Increasing Amounts of Coagulant

Ferric sulfate was added to six jars in amounts shown. Original turbidity of the water was 95 units. Dots indicate sampling points.

FIGURE 2

for the low turbidities of interest to the water plant. The direction of the effort is toward a standardized instrument geometry, and some standardized reference material.

II ZETA POTENTIAL MEASUREMENT

Particles suspended in water carry electrical charges at their surfaces. For particles in natural waters, the charge is usually negative as illustrated schematically in Figure 4. These electrical charges may arise through ionization of atoms at the particle surface, or particles may acquire the charge through adsorption of ions, particularly hydroxyl ions, from the water itself. These ions are tightly bound to the surface of the particle and attract ions of opposite charge from the mixture of positive and negative ions in the water. This layer of oppositely charged ions is also tightly held near the particle. As the force of attraction of ions decreases with increasing distance from the particle a diffuse layer is formed consisting of a varying mixture of positive and negative ions extending to such a distance from the particle that the mixture of ions is now similar to the composition present in the water. There is a plane of shear representing the portion of bound water and ions that will remain attached to the particle as it moves through the suspending liquid.

A consequence of the electrical double layer surrounding each particle in water is to create areas of electrical potential in a bulk solution that nominally has a zero potential. The electrostatic work required to transport a unit charge from this bulk solution through the phase boundaries of each layer surrounding a particle to any point measures the potential at that point. The potential increases sharply with increasing approach to the primary particle surface. The potentials of interest are illustrated in Figure 4. One is at the particle surface itself and measures the total potential of the double layer and is referred to as the Nernst potential. Another is at the boundary between the Stern diffuse layers and is called zeta potential (ζ). The zeta potential is interesting and important because unlike the Nernst potential an approximate zeta potential can be measured experimentally quite easily. It should be pointed out, however, that the zeta potential does not measure the total potential but rather a portion of it. It does not include the Stern layer.

The net effect of the existence of electrical double layers around particles is to inhibit the close approach of particles to each other and hence confers stability to the suspension. Both the thickness of the double layer and the surface charge density are sensitive to concentration and valence of the ions in solution, hence stability of the suspension can be markedly affected by adding suitable ions to the solution. The metal coagulants, alum, ferric sulfate, etc., added during treatment reduce these negative charges.

Measurement of zeta potential is another procedure useful for control of the coagulation process and for evaluating the results of the jar test. Zeta potential of particles is never determined directly, but is calculated from a measurement of particle electrophoretic mobility. In the usual procedure, a special apparatus is used with which the behavior of particles can be observed microscopically. Two such devices are shown in Figure 5. Under the influence of an applied voltage;

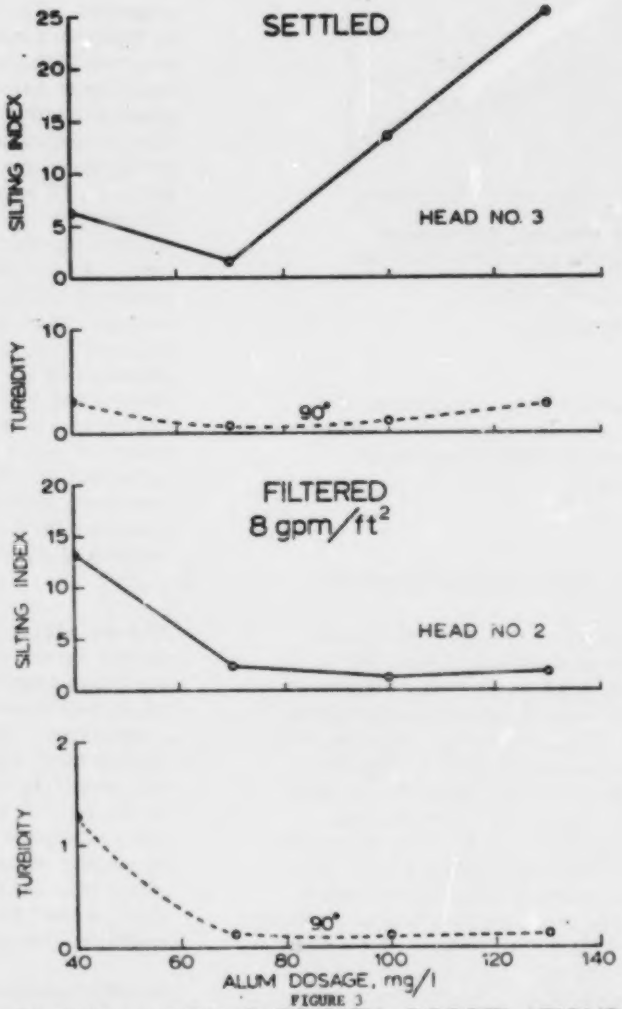


FIGURE 3

SILTING INDEX-TURBIDITY CORRELATIONS

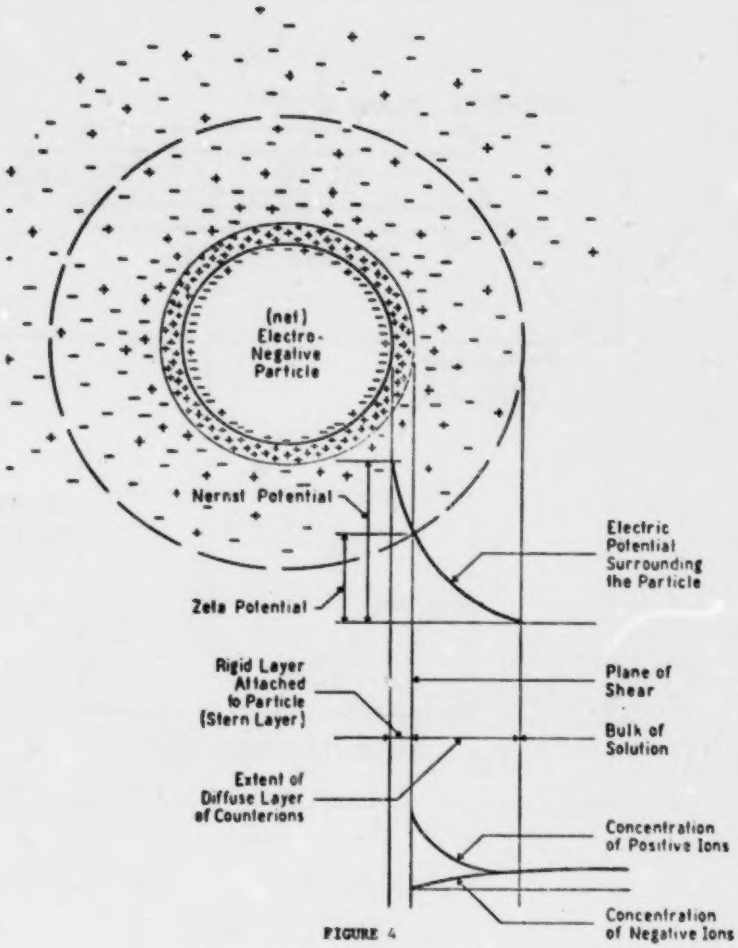

(net) Electro-Negative Particle
Nernst Potential
Zeta Potential
Electric Potential Surrounding the Particle
Rigid Layer Attached to Particle (Stern Layer)
Plane of Shear
Bulk of Solution
Extent of Diffuse Layer of Counterions
Concentration of Positive Ions
Concentration of Negative Ions

FIGURE 4

CONCEPT OF THE ZETA POTENTIAL

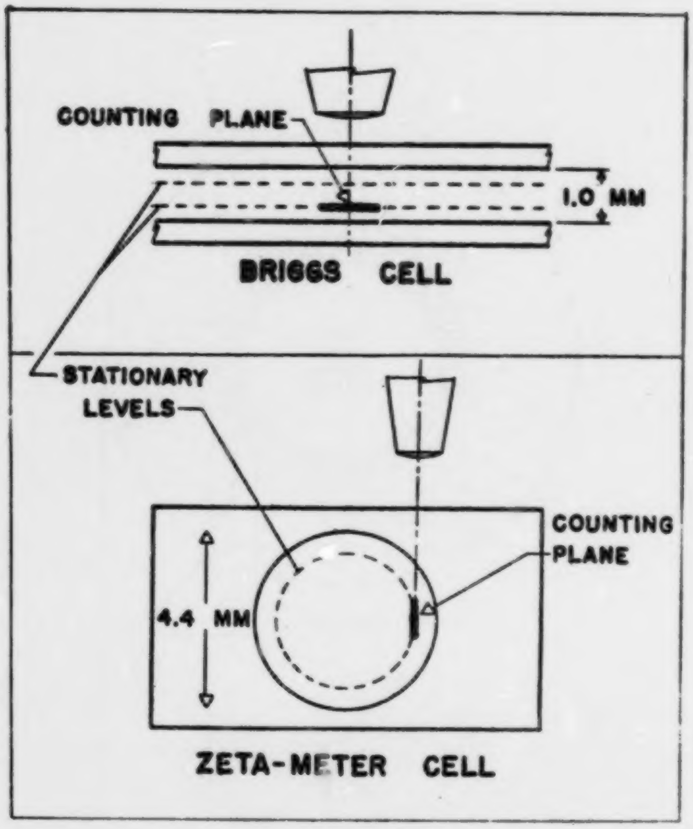

COUNTING PLANE
1.0 MM
BRIGGS CELL
STATIONARY
LEVELS
COUNTING
PLANE
4.4 MM
ZETA-METER CELL

FIGURE 5

SCHEMATIC OF CELL INTERIORS

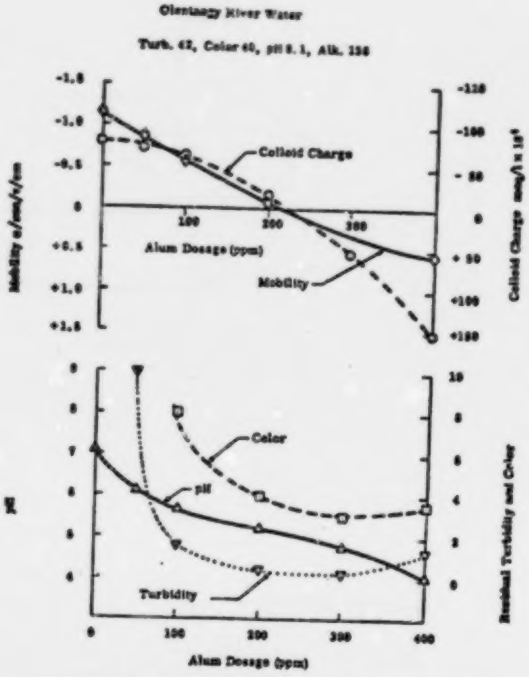
the charged particles will migrate to the poles of opposite polarity; the particle velocity can be measured by noting the time that it takes particles to traverse a known distance. From these and other data it is possible to calculate both electrophoretic mobility and zeta potential. The numerical value of the zeta potential that indicates optimum coagulation varies with the type of colloid to be removed from the water. Removal of organic colloids, such as color, is generally obtained at zeta potentials near zero, whereas optimum clay turbidity removals are obtained when zeta potentials are slightly negative ranging from -10 millivolts to perhaps -5 millivolts. But since a water may very likely contain a heterogeneous mixture of colloid types, the precise zeta potential which signals a good coagulation must be established for each water by other methods such as the jar test or by correlation to actual plant performance.

III COLLOID TITRATION

Electrophoretic mobility measurements determine the magnitude of repulsive forces of particles in water. They do not directly indicate the coagulant requirements unless a series of measurements are performed on a number of samples dosed with different quantities of coagulant. In colloid titration techniques, a single sample is dosed with coagulant until particle charges are neutralized, and an indicator which is sensitive to colloid charge changes color, Figure 6. The procedure is rapid but is subject to certain interferences. A streaming current detector, Figure 7, has been successfully used as an endpoint indicator with cationic polymers.

IV FILTERABILITY TECHNIQUES

Shull has determined a filterability number which is defined as the time required to filter a sample of supernatant from a specified jar test through a membrane filter divided by the time required to filter the same volume of distilled water under the same conditions. The filterability number is related to the alum dosage required by the plant, Figure 8.



- *Relationships between particle mobility, colloid charge, turbidity, color, and pH for Olentangy river water.*

FIGURE 6

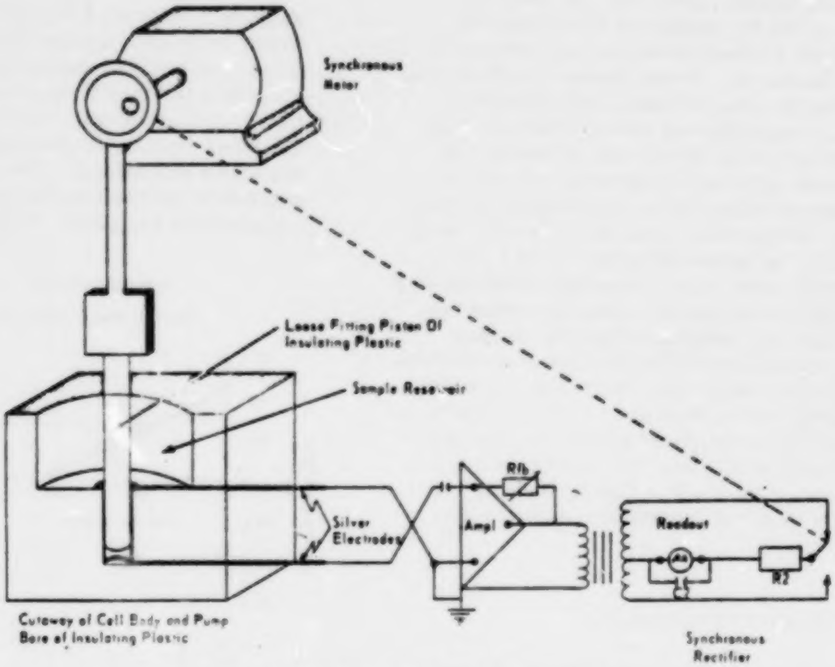

Synchronous Motor
Loose Fitting Piston Of Insulating Plastic
Sample Reservoir
Rfb
Silver Electrodes
Ampl
Readout
R2
Cutaway of Cell Body and Pump Bore of Insulating Plastic
Synchronous Rectifier

FIGURE 7. SIMPLIFIED DIAGRAM OF SCD INSTRUMENT

REFERENCES

1 Anon. Coagulation Control Tests, Jar Test Procedures, Water & Sew. Wks. Ref. & Data Ed., 102, No. 6, R-97. 1955.

2 Babcock, R.H. and Knowlton, K.F. Conductivity-Difference Control of Chemical Coagulation, JAWWA, 48, 622. 1956.

3 Baker, R.A., Campbell, S.J. and Anspach, F.R. Electrophoretic and Taste and Odor Measurement in Plant Control, JAWWA, 57, 363. 1965.

4 Baylis, J.R. Sensitive Detection of Suspended Matter, Public Works, 52, 6. 1922.

5 Baylis, J.R. Measuring the Volume of Coagulated Matter Passing Filter Beds, Water Works & Sewerage, 78, 33. 1931.

6 Bean, E.L., Campbell, S.J. and Anspach, F.R. Zeta Potential Measurements in the Control of Coagulation Chemical Dosage, JAWWA, 56, 214. 1964.

7 Black, A.P., Buswell, A.M., Eidsness, F.A. and Black, A.L. Review of the Jar Test, JAWWA, 49, 1414. 1957.

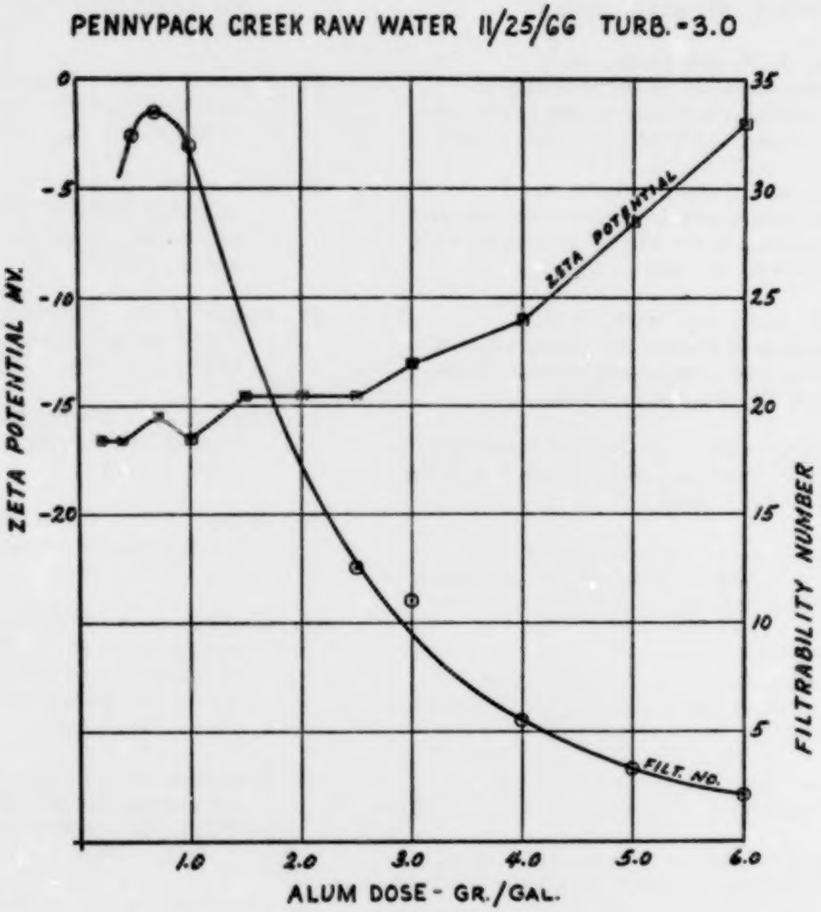

PENNYPACK CREEK RAW WATER 11/25/66 TURB. -3.0
ZETA POTENTIAL MV.
0
-5
-10
-15
-20
FILTRABILITY NUMBER
35
30
25
20
15
10
5
ZETA POTENTIAL
FILT. NO.
1.0
2.0
3.0
4.0
5.0
6.0
ALUM DOSE - GR./GAL.

FIGURE 8

8 Black, A.P. and Hannah, S.A. Measurement of Low Turbidities, JAWWA, 57, 901. 1965.

9 Black, A.P. and Smith, A.L. Determination of the Mobility of Colloidal Particles by Microelectrophoresis, JAWWA, 54, 926. 1962.

10 Black, A.P. and Smith, A.L. Improvements in Instrumentation and Techniques for Microelectrophoresis, JAWWA, 57, 485. 1965.

11 Black, A.P. and Smith, A.L. Suggested Method for Calibration of Briggs Microelectrophoresis Cells, JAWWA, 58, 445. 1966.

12 Buswell, A.M. Method for Automatic Control of Coagulant Dosage, JAWWA, 50, 479. 1958.

13 Buswell, A.M. and Larson, T.E. Optical Difficulties with the Cylindrical Cataphoresis Cell, J. Phys. Chem., 40, 833. 1936.

14 Coffman, P.A., Jr. Automation and Remote Control of Chemical Feeders, JAWWA, 49, 1378. 1957.

15 Cohen, J.M. Improved Jar Test Procedure, JAWWA, 49, 1425. 1957.

16 Conley, W.R., Jr. Integration of the Clarification Process, JAWWA, 57, 1333. 1965.

17 Flentje, M.E. and Wilson, P.S. Efficient Water Works Operation and the Purification Laboratory, JAWWA, 23, 7. 1931.

18 Granstrom, M. and Shearer, S.D. Conductometric Control of Coagulant Dosage in Treatment Plants, JAWWA, 50, 410. 1958.

19 Hall, E.S. The Zeta Potential of Aluminum Hydroxide in Relation to Water Treatment Coagulation, J. Appl. Chem., 15, 197. 1965.

20 Hannah, S.A., Cohen, J.M. and Robeck, G.G. Control Techniques for Coagulation-Filtration, JAWWA, 59, 9. 1967.

21 Hopkins, E.S. Coagulation Control with a Recording Potentiometer, JAWWA, 59, 9. 1967.

22 Kawamura, S. and Tanaka, Y. Applying Colloid Titration Techniques to Coagulant Dosage Control, Water & Sew. Works, 113, 384. 1966.

23 Knight, A.G. The Measurement of Turbidity in Water, J. Inst. Water Engrs., 4, 449. 1950.

24 Liepold, C. Mechanical Agitation and Alum Floc Formation, JAWWA, 26, 1070. 1934.

25 Nickel, J.B. Fundamental Techniques for Running Jar Tests, JAWWA, 37, 1207. 1945.

26 Ockershausen, R.W. Coagulation Symposium, Pt. 1, Process Control in Plants Using Aluminum Sulfate, Water Works and Wastes Engr., 2, No. 1, 53. 1965.

27 Packham, R.F. The Absorptiometric Determination of Aluminum in Water, Proc. Soc. Water Treat. and Exam., 7, 102. 1958.

28 Packham, R.F. A Multiple Stirrer Unit for Coagulation Tests, Water & Water Engr., 66, 105. 1962.

29 Palin, A.T. Photometric Determination of Color and Turbidity of Water, Water & Sew. Works, 104, 492. 1957.

30 Riddick, T.M. A Stirring Apparatus for the Determination of Coagulant Doses, Water Works & Sewerage, 81, 358. 1934.

31 Riddick, T.M. Zeta Potential: New Tool for Water Treatment Pt. 1, Chem. Engr., 68, 13:121. 1961.

32 Riddick, T.M. Zeta Potential and its Application to Difficult Waters, JAWWA, 53, 1007. 1961.

33 Riddick, T.M. Role of Zeta Potential in Coagulation Involving Hydrous Oxides, Tappi, 47, 171A. 1964.

34 Rock, R.M. and Burbank, N.C., Jr. Improved Briggs Microelectrophoresis Cell and Comparisons with the Riddick Cell, JAWWA, 58, 676. 1966.

35 Rose, H.E. The Analysis of Water by the Assessment of Turbidity, J. Inst. Water Engrs., 5, 521. 1951.

36 Shull, K.E. Filterability Techniques for Improving Water Clarification, JAWWA, 59, 9. 1967.

37 Tolman, S.L. Use of Models in Solving Flocculation Problems, JAWWA, 41, 641. 1949.

38 Vaughn, J.C. Coagulation Symposium V, Inplant Evaluation and Control of Coagulation is One Tool for Better Service, Water Works & Wastes Engr., 2, No. 6, 100. 1965.

39 Williams, R.L. Microelectrophoretic Studies of Coagulation with Aluminum Sulfate, JAWWA, 57, 801. 1965.

40 Woxholt, W.G. Electrical Conductivity and the Stability of Colloids, JAWWA, 51, 885. 1959.

41 O'Melia, C.R. A Review of the Coagulation Process, Public Works, p. 87. May 1969.

42 Kawamura, S. Removal of Color by Alum Coagulation-The Application of Colloid Titration Techniques to Coagulant Dosage Control, Water & Sew. Works, 114, 282-324. 1967.

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COMPARATIVE METHODOLOGY AND QUALITY CONTROL

I DEFINITION OF AN INTERLABORATORY STUDY

A What it is not.

It is not an interlaboratory evaluation of materials, or reagents.

It is not an interlaboratory evaluation of different conditions to test.

It is not an initial study of a method.

It is not a study to develop methods.

B What it is.

It is a between-laboratory evaluation of exact physical, chemical or biological methods which yields a measurement of desired properties.

II PURPOSES OF INTERLABORATORY STUDY

A Selection of an Analytical Method.

B Evaluation of a Selected Analytical Method.

C Evaluation of Laboratory and Analyst Performance.

III PREPARATION FOR INTERLABORATORY STUDY

A Laboratories must be in control. Between-replicate deviation should be very small in all laboratories.

B Ruggedness testing of a method has been completed by the method developer, or by a single qualified laboratory (effects of small changes in time, temperature, pH, reagent and sample volume measurements, etc. are known)[1].

C All laboratories and analysts have used the method to be tested. Any special equipment or reagents required are known and available. If all analysts are not acquainted with the method, a simple preliminary study is undertaken to accomplish this[2].

IV THE TEST METHOD WRITE-UP

A Exact method write-ups must be provided to each analyst. The technical description of an analytical method is difficult.

B The language and organization must be complete, yet simple and unambiguous. These characteristics are often conflicting.

C Requirements in a method may seem trivial, yet if these are not recognized and controlled, the entire study can be useless.

V WRITTEN INSTRUCTIONS FOR THE STUDY

A Explicit directions for sample preservation, sample make-up, time limitations, sequence of analyses, etc. are provided.

B Advance notice of tests is given so that laboratories can integrate the test into their program and realistic deadlines can be established.

C Once established deadlines for agreement to participate and completion of analyses and reporting should be followed closely.

VI THE TEST SAMPLE

A The sample is carefully designed to reflect the concentrations found in natural samples and yet is within the best portion of the concentration range for the method.

B Since precision of almost all methods varies with concentration, a comprehensive study must include several levels of concentration.

C Since accuracy of a method is very important, exactly known levels of constituents are added both to distilled and natural waters for testing. Stabilized natural waters as samples are not used.

VII SAMPLE FORM AND CONTAINER

A Samples can be prepared as dilute simulated or natural water samples to be analyzed, as is. These samples have an advantage in that they reach the analyst exactly as a routine sample. There is no error in making a dilution and this source of variance in the method is removed. Its disadvantages are: the serious logistics problem from storage of any large number of these samples, a relatively high cost of mailing, and a limited choice of preservation methods because of the size of containers and probable use of plastic containers.

B Samples can be prepared as concentrates for final dilution at the testing laboratory. Advantages are the greatly reduced space requirements and subsequently a greatly reduced cost of mailing. With use of sealed glass ampuls, preservation of concentrates is easily obtained through steam sterilization. Chemical preservatives can be used at fairly high levels in the concentrate and be removed as interferences by the dilution of the concentrate to final sample volume. Theoretically, a well-designed sample properly sealed in glass is stable indefinitely.

VIII INTERLABORATORY STUDY DESIGN

A Selection of sample size and number of replicates.

There are a number of formulas or calculations involving estimate deviation and allowable change for error which will assist you in estimating the minimum number of replicates required in a study. Two examples follow:

1 Sample Size, $\underline{n} = \left(\frac{3\sigma'}{E}\right)^2$

σ' = advance estimate of the standard deviation of the method.

E = the maximum allowable difference between the estimate to be made from the sample and the result of testing all the units in the universe.

3 = a factor corresponding to a probability of about 3 parts/1000 that the difference between the sample estimate and the result of testing all units in the universe is greater than E. The choice of the factor 3 is recommended for general use. With the factor 3, and with a universe standard deviation equal to the advance estimate, it is "practically certain" that the sampling error will not exceed E. There are occasions, however, where a lesser degree of certainty is desired, which a smaller factor provides.[7]

B Small sample statistics

Although there is an increasing tendency to treat smaller numbers of samples statistically to arrive at a data evaluation, interlaboratory tests have as their main objective, the study of variability. For reliable estimates of variability, relatively large samples are required. Mandel suggests that when the number of laboratories is small, analysts using completely independent sets of equipment can be treated as separate collaborating laboratories[3].

IX ASTM DESIGNS FOR ONE OR MORE MATERIALS

A Highly developed test designs are available such as that in ASTM Publication 335[6]. This study has the general design: Total measurements = $\underline{p}$ laboratories X $\underline{q}$ materials X $\underline{n}$ replicates.

B If no further grouping is made, the n measurements within a cell are called replicates. The ASTM Committee considers that these replicates constitute a random sample from a single statistical population. If the $\underline{n}$ measurements in a cell can be identified further by operators, days of test, run, etc., the factors may be nested within each other or they may form a crossed classification. Control charts are constructed for the desired factors using 2 σ control limits based on variation in replicates.

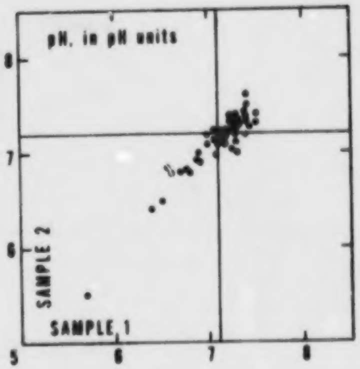
X YOUDEN STUDY DESIGN

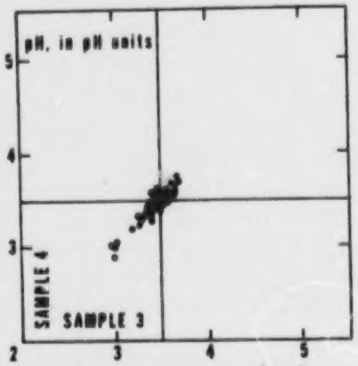
A Youden's proposed a no-replicate interlaboratory study which is used in **WQO**[4]. Pairs of sample of similar concentrations (x, y samples) are prepared at each of several levels. Statistics are derived on each sample as shown in Figure 1.

B Methods are evaluated using x, y charts prepared for each pair of samples. Data form ellipses around the true value because of systematic error. Random error is shown as the distance from the 45° slope. General scatter indicates poor method precision. Bias is shown by movement of the ellipse away from the true values. Examples are shown in Figures 1, 2, and 3.

TWO-SAMPLE CHARTS & METHOD STATISTICS FOR pH METHOD, FWPCA

STATISTIC	SAMPLE					
	1	2	3	4	5	6
TRUE VALUE, pH units	7.1	7.2	3.5	3.5	8.0	8.0
ARITHMETIC MEAN OF REPORTED VALUES, pH units	7.17	7.19	3.48	3.49	7.98	8.01
ACCURACY AS RELATIVE ERROR (% BIAS)	+1.01	-0.03	-0.29	-0.00	-0.12	+0.16
STANDARD DEVIATION, pH units	0.20	0.18	0.10	0.11	0.13	0.12
95% CONFIDENCE INTERVAL pH units	6.78- 7.56	6.84- 7.54	3.28- 3.68	3.27- 3.71	7.72- 8.24	7.78- 8.24





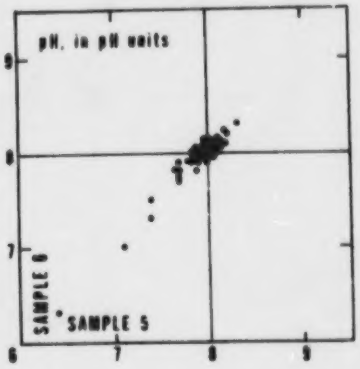
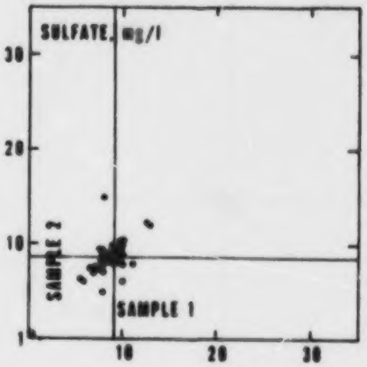


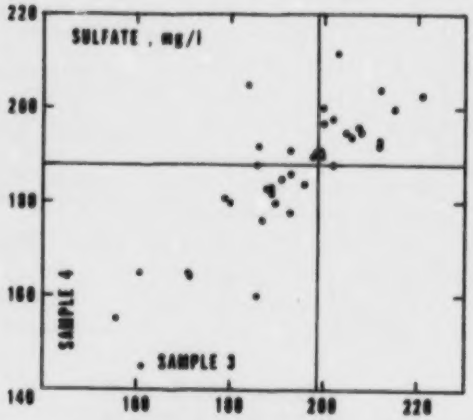
Figure 1. GOOD PRECISION AND ACCURACY

From: *FWPCA Method Study 1, Mineral and Physical Analyses*[5]

TWO-SAMPLE CHARTS & METHOD STATISTICS FOR SULFATE METHOD, FWPCA

STATISTIC	SAMPLE					
	1	2	3	4	5	6
TRUE VALUE, mg/l	9.2	8.6	199	188	122	110
ARITHMETIC MEAN OF REPORTED VALUES, mg/l	8.44	8.28	196	188	118	107
ACCURACY AS RELATIVE ERROR (% BIAS)	-8.26	-3.72	-1.70	+0.04	-3.37	-3.01
STANDARD DEVIATION, mg/l	1.70	2.30	11.8	9.50	7.50	7.80
95% CONFIDENCE INTERVAL, mg/l	4.96-11.9	3.77-12.8	172-219	169-208	103-133	91.3-122





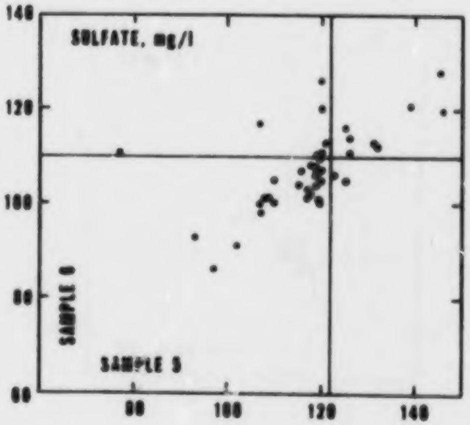
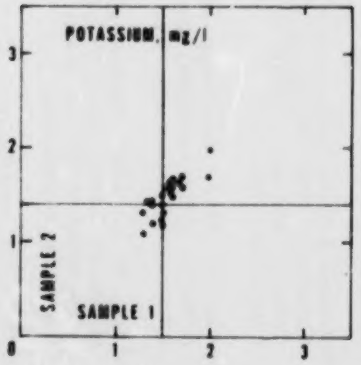


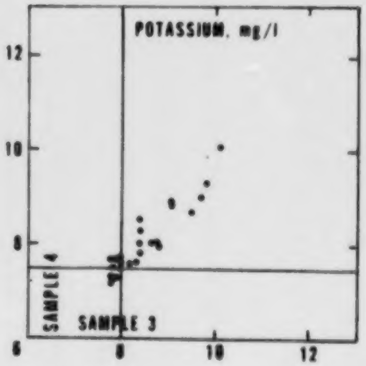
Figure 2. FAIR ACCURACY, POOR PRECISION

From: *FWPCA Method Study 1, Mineral and Physical Analyses*[5]

TWO-SAMPLE CHARTS & STATISTICS
FOR POTASSIUM, FWPCA METHOD

STATISTIC	SAMPLE					
	1	2	3	4	5	6
TRUE VALUE	1.5	1.4	8.0	7.5	20.0	19.0
ARITHMETIC MEAN OF REPORTED VALUES, mg/l	1.57	1.49	8.60	8.14	21.5	20.4
RELATIVE ERROR (% BIAS)	+4.76	+6.63	+7.58	+8.66	+7.36	+7.37
STANDARD DEVIATION	0.17	0.22	0.64	0.66	1.11	1.50
95% CONFIDENCE INTERVAL	1.24- 1.90	1.06- 1.92	7.35- 9.85	6.85- 9.43	19.3- 23.6	17.3- 23.5





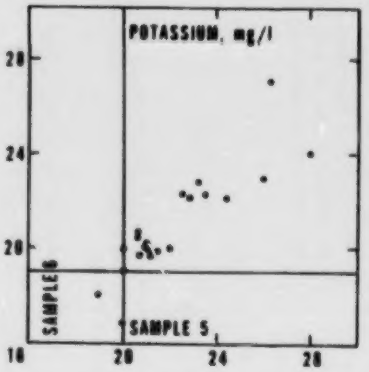


Figure 3. POOR ACCURACY (BIAS)

From: *FWPCA Method Study 1, Mineral and Physical Analyses*[5]

C Youden's precision measure

Youden assumes that the systematic error is the same for the x and y samples. Calculation of (x - y) differences drops out the systematic error of a participant and only random error remains in the difference (D). By calculating the standard deviation of the differences of all laboratories, a precision calculation is possible for the test:

$$S_r = \sqrt{\frac{\Sigma(D_i - \bar{D})^2}{2(n - 1)}} \; (n - 1) \text{ df.}$$

Where the divisor 2 is introduced because the D values are differences between two measurements.

The standard deviation of the data is calculated by T, the total of the paired X and y results:

$$S_d = \sqrt{\frac{\Sigma(T_i - \bar{T})^2}{2(n - 1)}}$$

D WQO precision measure

Method precision is reported for each sample since it varies with concentration. Analyst precision is measured as the difference in the error term of the method and the error term of analyst derived from an analysis of variance. Whenever the error term for the analyst exceeds that of the method, a review of lab procedures is in order.

XI INTERLABORATORY STUDY REPORT

A Reports are slanted toward audience.

B All data are coded by laboratory and analyst.

C Glossary of terms is necessary.

D Computer use increases statistical capabilities tremendously. There is a danger of "over-evaluation".

E Ultimate efficiency in reporting data is the use of print-out directly as text of a report[8].

REFERENCES

1 Youden, W. J. 1961, Experimental Design and ASTM Committees. Materials Research & Standards, 1(11):862-867.

2 Preliminary Study, Nutrient Analyses, September 1969. Analytical Quality Control Laboratory, FWPCA, Cincinnati, OH.

3 Mandel, J. 1964. The Statistical Analysis of Experimental Data. Interscience Publishers, New York.

4 Youden, W. J. 1967. Statistical Techniques for Collaborative Tests. The Association of Official Analytical Chemists, Washington, D. C.

5 Winter, J. A. and Midgett, M. R. 1969. FWPCA Method Study 1, Mineral and Physical Analyses, Analytical Quality Control Laboratory, FWPCA, Cincinnati, OH.

6 ASTM Manual for Conducting an Interlaboratory Study of a Test Method, American Society for Testing and Materials, Philadelphia, PA, 1963.

7 ASTM Manual on Quality Control of Materials, STP 15-C, American Society for Testing and Materials, Philadelphia, PA, 1951.

8 Larson, Kenneth E., The Summarization of Data, J. of Quality Technology, Vol. 1, No. 1, Jan. 1969.

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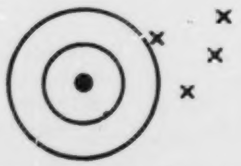
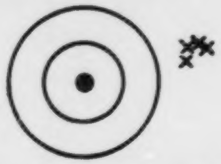
ACCURACY-PRECISION-ERROR

I INTRODUCTION

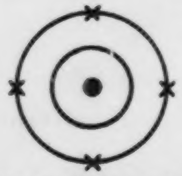
An analytical method is subject to errors. These errors may affect the accuracy of the method because they introduce bias into the results. There are other types of errors which affect the precision of the method because they produce random fluctuations in the data. The most desirable situation for the analyst is shown in the diagram in Figure 1 where the results are both precise and accurate.



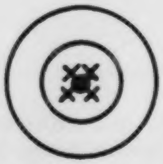
IMPRECISE AND INACCURATE



PRECISE BUT INACCURATE



ACCURATE BUT IMPRECISE



PRECISE AND ACCURATE

Figure 1. PRECISION AND ACCURACY

A Accuracy

For results to be accurate, the analysis used must give values close to the true value.(See Figure 1)

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. It is a measure of the degree to which results "check." (See Figure 1)

C Note

It is possible to have precision without accuracy. (See Figure 1)

II DETERMINATE ERROR AND ACCURACY

A determinate error is one which contributes a constant error or bias to results, causing them to be inaccurate. This constant error makes it possible for results to agree with each other (be precise) and still be inaccurate.(See Figure 1)

Determinate errors have "assignable" causes which can usually be identified and either eliminated or controlled. (The terms "determinate" error, "assignable" error, and "systematic" error are synonymous).

A Sources of Determinate Error

1 Method error

Method errors are those that are inherent in the procedure. These are very serious and the hardest to detect and correct. The most common method error is the presence of interferences in the sample. Other examples would be precipitation of substances other than the desired material, partial solubility of precipitates, and entrainment as in a solvent extraction procedure.

2 Personal errors

Personal errors are attributable to individual mistakes which are consistently made by an analyst. These errors are the result of consistent carelessness, lack of knowledge or personal bias. Examples are errors in calculations, use of contaminated reagents, non-representative sampling, or poor calibration of standards and instruments.

3 Instrumental errors

Instrumental errors are those which are caused by an analytical instrument or by the effects of the environment acting on the instrument. Moisture in a G.C. column, improper wavelength markings on a spectrophotometer or incorrect scoring on a buret would be examples.

B Effects of Determinate Error

1 Additive

An additive determinate error is one which has a constant value regardless of the amount of analytically sought constituent present in the sample. (See Figure 2)

2 Proportional

A proportional determinate error changes value according to the amount of analytically sought constituent in the sample. (See Figure 3)

III DETECTION OF DETERMINATE ERROR

A Spiked Samples

1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

a Mean error - the difference between the mean of the data and the true result.

b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.5 mg/l. He then adds 1 mg/l of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample, and calculation of both mean and relative errors.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

X_i
1.55 mg/l
1.58
1.60
1.47
1.35

1 Calculation of mean error

$$\bar{X} = \frac{7.55}{5} = 1.51 \text{ mg/l}$$

Mean error = +0.01 mg/l

2 Calculation of relative error

$$\text{Relative Error} = \frac{0.01 \times 100}{1.50} = 0.7\%$$

2 Control charts

Trends and shifts on control charts may also indicate determinant error. Using spiked samples, the standard deviation is calculated and control limits (usually $\pm$ 3 standard deviations) for the analysis are set (see Figure 4. For further discussion of control limits, see reference 3, p. 62.

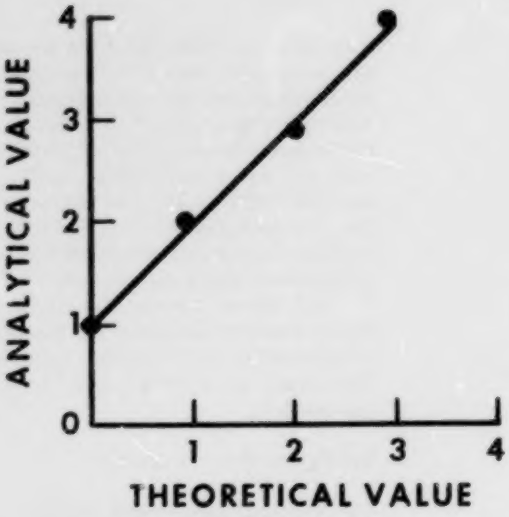


Figure 2. ADDITIVE ERROR

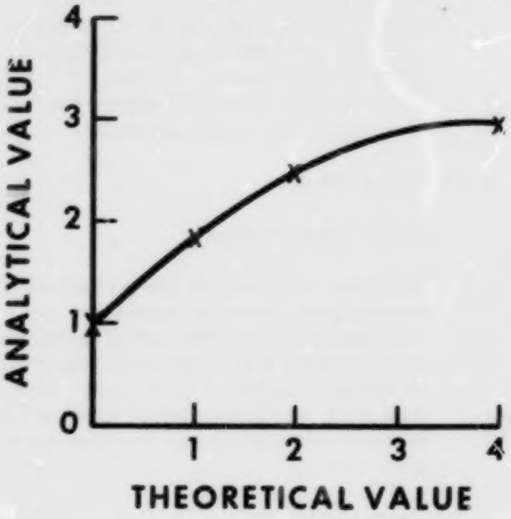


Figure 3. PROPORTIONAL ERROR

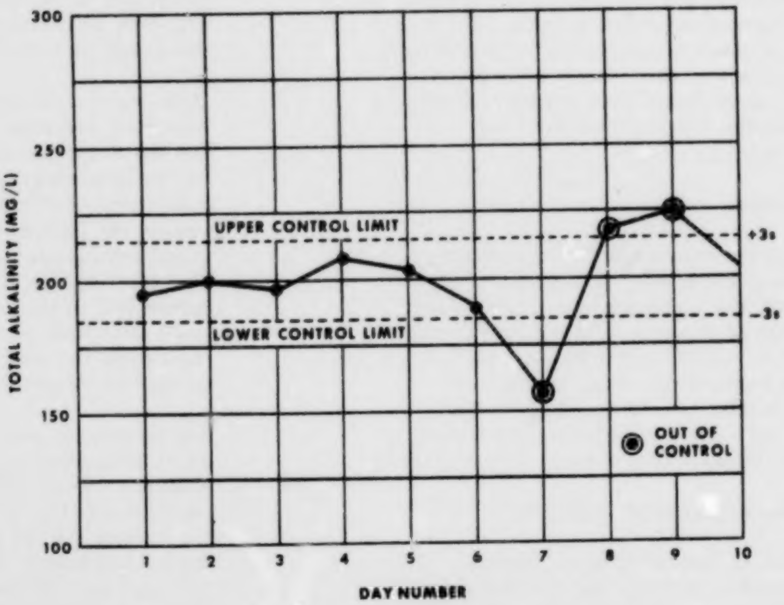


Figure 4. CONTROL CHART

3 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden[11] provides excellent techniques for detecting bias in this situation.

B Unknown Samples

1 Independent method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error. However, another reliable method may not be available or may be laborious to perform.

2 Control charts

It is possible to plot a control chart (Figure 4) even when it is not possible to spike a sample. One can use as a reference value an average of a series of replicate determinations performed on a composite check sample. Such a sample must be preserved or stabilized in such a way that the concentration of the constituent being measured will not change from day to day (see reference 1).

3 Aliquoting

If the determinate error is additive, the magnitude may be estimated by plotting the measured quantity versus a range of sample volumes or sample weights. If the error has a constant value regardless of the amount of analytically sought constituent, then a straight line fitted to the points will not go through the origin. (See Figure 5)

4 Youden's graphical technique

Dr. W. J. Youden has devised an approach to test for determinant errors with a minimum of effort on the part of the analyst and his laboratory.

a Technique

Two different test samples (X and Y) are prepared and distributed for analysis to as many individuals or laboratories as possible. Each participant is asked to perform only one determination on each sample (NOTE: It is important that the samples be relatively similar in concentration of the constituent being measured). Table 2 shows the results on two such samples analyzed for percent potassium by 14 different laboratories. The mean for each sample has been calculated.

b Interpretation - Figure 6

The vertical line drawn on the graph represents the mean $(\overline{X})$ of all the results obtained on Sample X; the horizontal line was drawn through the mean $(\overline{Y})$ of all the results obtained on sample Y. Each pair of laboratory results can then be plotted as a point on the graph (marked X. 1 etc.)

If the ratio of the bias (error) to standard deviation is close to zero for the determinations submitted by the participants, then one would expect the distribution of the paired values (or points) to be close to equal among the four quadrants. The fact that the majority of the points fall in the (+, +) and (-, -) quadrants indicates that the results have been influenced by some source of bias or determinate error.

Furthermore, one can even learn something about a participant's precision from the graph. If all participants had perfect precision (no indeterminate error), then all the paired points would fall on a 45° line passing through the origin. Consequently the distance from such a 45° line of each participant's point provides an indication of that participant's precision.

The Youden method is also discussed in this manual in the section on Comparative Methodology and Quality Control. A quantitative treatment of this subject can be found in references 11 and 12.

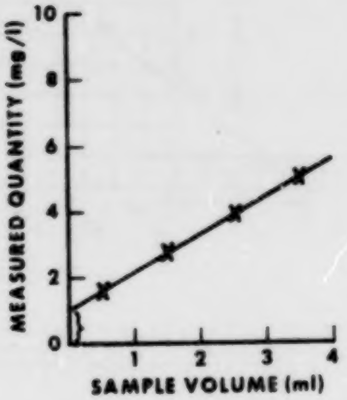


Figure 5.

Table 2

Laboratory	Sample X	Sample Y
1	9.74%	8.50%
2	9.92	8.28
3	9.98	8.84
4	9.99	8.24
5	10.00	8.73
6	10.11	8.54
7	10.12	8.64
8	10.14	8.82
9	10.19	9.04
10	10.23	8.93
11	10.25	8.97
12	10.29	8.80
13	10.55	9.21
14	10.62	8.95

$\overline{X} = 10.15$ $\overline{Y} = 8.75$

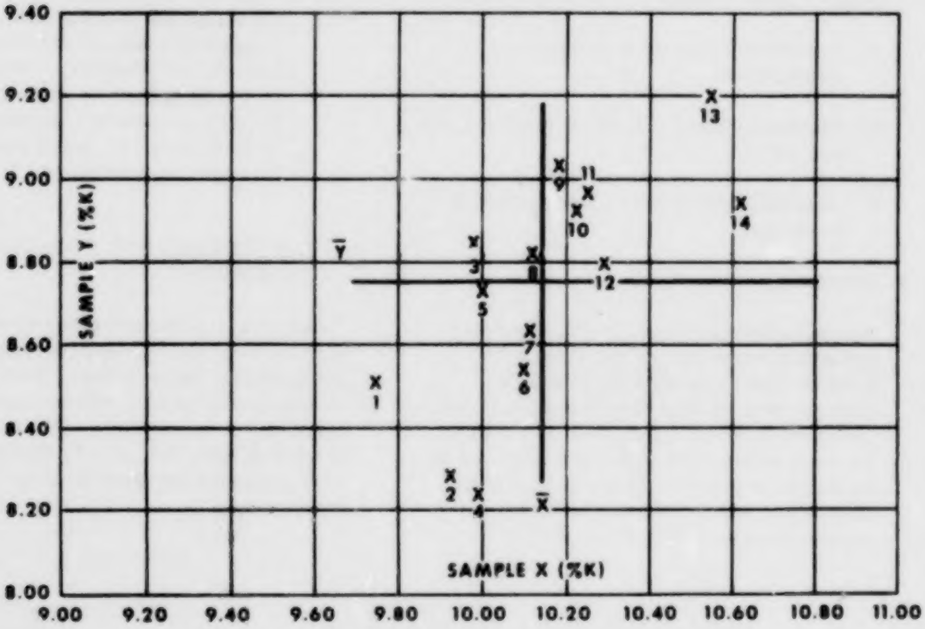


Figure 6. YOUDEN'S GRAPHICAL TECHNIQUE

IV ELIMINATION OF DETERMINATE ERROR

Various approaches can be used to eliminate the source of determinate error. The approach used depends upon whether the source is personal, method, or instrumental.

A Personal

Great care must be used to avoid producing an unconfident attitude in a technician. It is undesirable for the analyst to feel he is being "policed."

B Method and Instrumental

1 Blanks

Blanks can be used to correct for interferences from reagents, sample color, etc.

2 Correction factors

Examples of correction factors used in environmental analyses to eliminate determinate errors are the following:

a Recovery factors in organic extractions

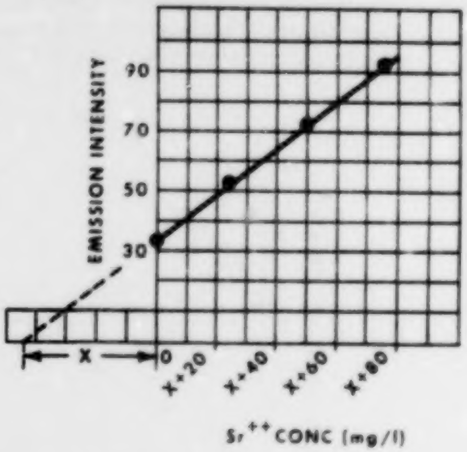
b Chemical yield values in gravimetric analyses

c Counting efficiencies for radiation counters

3 Standard addition

Sample interferences producing determinate errors can be overcome by adding equal amounts of unknown sample to a series of standards. The concentration of the unknown can then be determined graphically from a plot of the measured quantity (absorption, emission, etc.) versus the standard concentration. (See Figure 7)

Figure 7. METHOD OF STANDARD ADDITION GRAPHICAL METHOD OF COMPUTING Sr CONCENTRATION



NOTE: The X value appearing along the abscissa (i.e., X + 20, X + 40, etc.) refers to the unknown added to the strontium standard

4 Standard compensation

Another approach is to prepare the standard so that its composition resembles that of the sample as closely as possible. The objective of the approach is exactly the same as that of standard addition - to compensate for the presence of interfering substances in the unknown.

V INDETERMINATE ERROR AND PRECISION

Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Such variation in results is due to indeterminate error, also known as random, chance or uncontrollable error. Indeterminate error affects the precision or agreement among results.

A Sources of Indeterminate Error

Indeterminate errors are due to unassignable or "chance causes." Examples are inadvertent contamination of sample or glassware, variation in reagent additions, or variations in instrument response (see Figure 8).

B Effects of Indeterminate Error

Since the causes of these errors are random, the effects are also random. Fortunately, these random variations conform to the "Laws of Chance" so statistical measures of precision can be used to quantitate indeterminate errors.

VI DETECTION OF INDETERMINATE ERROR

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

An estimation of indeterminate error can be obtained through a calculation of the standard deviation. The following formulas should be applied to random data which follows a normal distribution. Normality can be checked by ranking and plotting the data on normal probability paper; it should fall on a straight line (see Figure 9). Any values which do not fall close to the straight line (those values encircled) should be rejected in the calculation of standard deviation. Other statistical tests (8) can be used to objectively evaluate the rejection of outliers. The value obtained for standard deviation for a particular method may vary with the analyst, the concentration range of the constituent, and the composition of the sample analyzed. The confidence of the estimate is increased as the number of results (n) used to compute the standard deviation is increased.

1 Replicate results on the same sample:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n-1}} \qquad (1)$$

X_i = value of single result

$\overline{X}$ = average (mean) of results on same sample

n = number of results

Example: Table 3 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a colaborative study. Calculate the standard deviation of these results.

$\overline{X}$ = 192 mg/l

n = 36.0

$\Sigma(X_i - \overline{X})^2$ = 58,200

$$s = \sqrt{\frac{58,200}{35}}$$

s = 41 mg/l

2 Duplicate results on different samples

In a laboratory where duplicates are routinely run, it would be simple to use the following formula for evaluation of standard deviation.

$$s = \sqrt{\frac{\Sigma(d^2)}{2k}} \quad \text{See reference 5, page 654)} \qquad (2)$$

d = difference between duplicates

k = number of samples

Example: Table 4 contains a set of phosphate results obtained on aqueous samples in the range 100-200 mg/l. Calculate the standard deviation of these results.

$\Sigma(d^2) = 16$

$k = 22$

$s = \sqrt{\frac{16}{44}}$

$s = .61$ mg/l

3 Duplicate and triplicate results on different samples

$$s = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - k}} \quad \text{(See reference 7, page 73)} \qquad (3)$$

Note: $\bar{X}$ = average of results on the same sample

Example: Table 5 contains a set of % nitrogen results obtained on unknown organic compounds.

Calculate the standard deviation.

$\Sigma(X_i - \bar{X})^2 = .2401$

$n - k = 22$

$s = \sqrt{\frac{.2401}{22}}$

$s = .104\%$

4 Use of range to estimate standard deviation

For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (See Table 6).

$$\frac{R}{d_N} = s \qquad (4)$$

Example: Table 7 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formula (1)

$\bar{X} = 0.72$ mg/l

$n = 5$

$\Sigma(X_i - \bar{X})^2 = .0134$

$s = \sqrt{\frac{.0134}{4}}$

$s = .058$ mg/l

b Use of formula (4)

$R = .14$ mg/l

$d_N = 2.33$

$s = \frac{.14 \text{ mg/l}}{2.33}$

$s = .060$ mg/l

C Coefficient of Variation

An estimation of determinate error can also be made by calculating the coefficient of variation.

$$V = \frac{s}{\bar{X}} \times 100$$

By comparing the standard deviation (dispersion) to the average or mean value (central tendency) in a set of data and expressing this relative standard deviation as a percentage, the analyst has a meaningful interpretation of the degree of dispersion present. Indeterminate error would be the cause of undue dispersion.

Example: Table 3

$s = 41$ mg/l

$\overline{X} = 192$ mg/l

$$V = \frac{41}{192} \times 100$$

$V = 21\%$

ACKNOWLEDGEMENT:

This outline contains certain portions of previous outlines by Betty A. Punghorst, former Chemist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

REFERENCES

1 Accuracy in Clinical Chemistry. Dade Reagents, Inc., Miami, Florida.

2 Allan, Douglas H. Statistical Quality Control. Reinhold Publishing Corp., New York. 1959.

3 American Society for Testing Materials ASTM Manual on Quality Control of Materials. Special Technical Publication 15-C. 1951.

4 Bauer, E.L. A Statistical Manual for Chemists. Academic Press, New York. 1960.

5 Bennett, Carl A. and Franklin, Norman L. Statistical Analysis in Chemistry and the Chemical Industry. John Wiley & Sons, Inc., New York. 1954.

6 Chase and Rabinocvitz. Principles of Radioisotope Methodology. Minneapolis Burgess Publishing Company. 1964.

7 Mickley, Harold S., Sherwood, Thomas K. and Reed, Charles E. Applied Mathematics in Chemical Engineering. McGraw-Hill Book Company, New York. 1957.

8 Natrella, M.G. Experimental Statistics, National Bureau of Standards Handbook 91. U.S. Dept. of Commerce. 1963.

9 Schickner, Frank A. Personal Communication. The Procter & Gamble Company, Miami Valley Laboratories, Research & Development Department, P.O. Box 39175, Cincinnati, OH 45239.

10 Youden, W.J. The Collaborative Test. JAOAC 46:55-62. January 1963.

11 Youden, W.J. The Sample, The Procedure, and the Laboratory. Anal. Chem. 32:23-37A. Dec. 1960.

12 Youden, W.V. Statistical Techniques for Collaborative Tests. Association of Official Analytical Chemists, Box 540, Benjamin Franklin Station, Washington, DC. 1967.

This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, Water Quality Office, EPA, Cincinnati, OH. 45226.

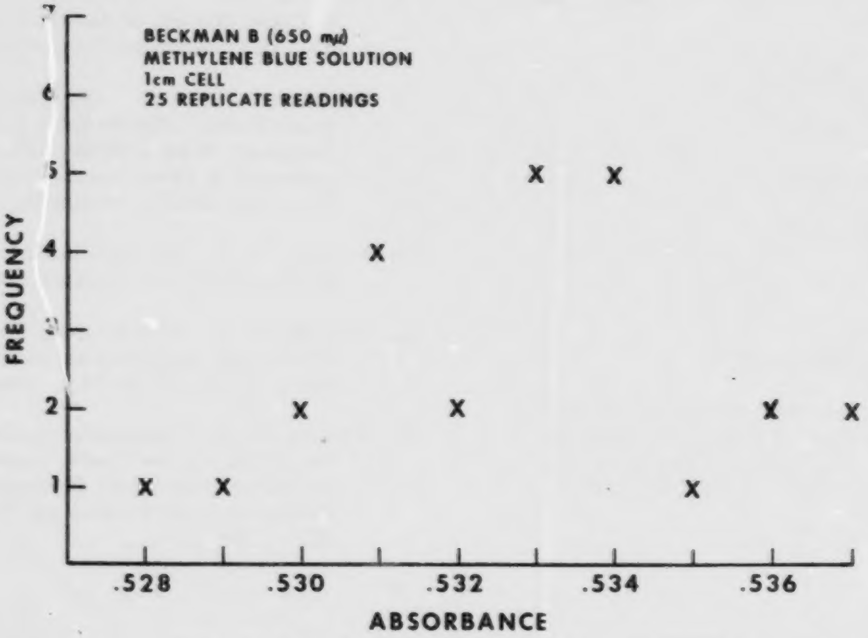


Figure 8. VARIATIONS IN SPECTROPHOTOMETER READINGS

FIGURE 9 NORMAL PROBABILITY CURVE

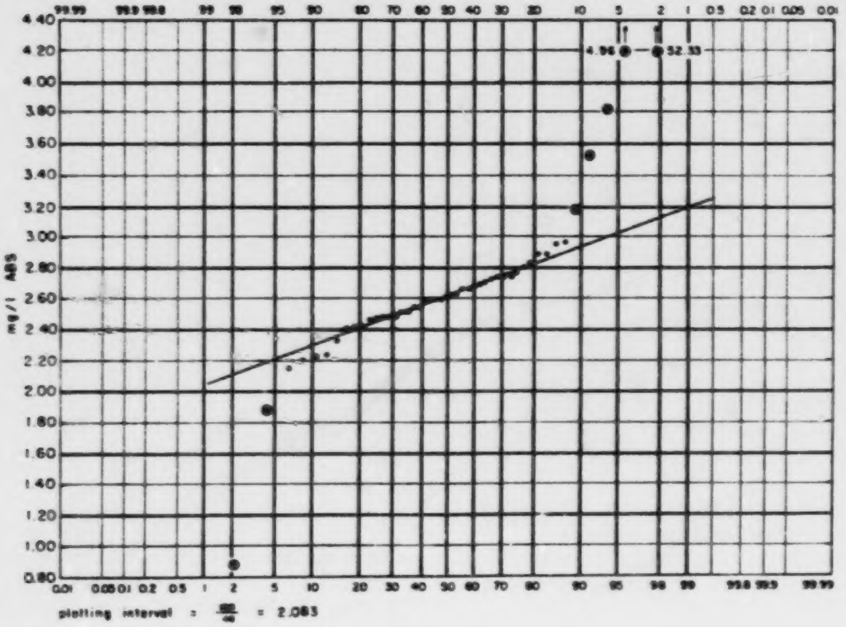


Table 3

SAMPLE: 150 mg/l glucose + 150 mg/l glutamic acid (1% dilution)
DETERMINATION: 5-day Biochemical Oxygen Demand

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
100 mg/l	-92 mg/l	8464 (mg/l)^2	198 mg/l	6 mg/l	36 (mg/l)^2
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	- 3	9	238	46	2116
190	- 2	4	247	55	3025
196	4	16	250	58	3344
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R. A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Table 4*

SAMPLE: Aqueous
CONCENTRATION RANGE: 30 - 70 mg/l
DETERMINATION: Phosphate (Lucerna, Conde, and Prat Method)

Sample	Mg/l P	d	d^2	Sample	Mg/l P	d	d^2
A	51 53	2	4	L	54 56	2	4
B	39 39	0	0	M	36 37	1	1
C	53 54	1	1	N	52 52	0	0
D	47 47	0	0	O	58 58	0	0
E	50 51	1	1	P	54 54	0	0
F	48 47	1	1	Q	54 54	0	0
G	50 50	0	0	R	48 48	0	0
H	47 47	0	0	S	52 51	1	1
I	42 42	0	0	T	53 53	0	0
J	50 51	1	1	U	46 47	1	1
K	59 60	1	1	V	42 42	0	0

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 5*

SAMPLE: Unknown Organic Compounds
DETERMINATION: % Nitrogen (Kjeldahl)

CONCENTRATION
RANGE: 10% - 20%

Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
A	16.48	16.47	.01	.0001
	16.45		.01	.0001
B	16.50	16.50	0	0
	16.49		.01	.0001
C	16.72	16.65	.07	.0049
	16.57		.08	.0064
D	17.52	17.58	.06	.0036
	17.60		.02	.0004
	17.63		.05	.0025
E	16.31	16.31	0	0
	16.30		.01	.0001
F	16.40	16.35	.05	.0025
	16.31		.04	.0016
	16.35		0	0
G	17.56	17.55	.01	.0001
	17.54		.01	.0001
H	14.96	14.81	.15	.0225
	14.66		.15	.0225
I	19.15	19.02	.13	.0169
	18.89		.13	.0169

Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
J	13.93	13.75	.18	.0324
	13.56		.19	.0361
K	10.34	10.27	.07	.0049
	10.19		.08	.0064
L	17.16	17.15	.01	.0001
	17.13		.02	.0004
M	15.01	15.03	.02	.0004
	15.05		.02	.0004
N	12.44	12.62	.18	.0324
	12.70		.08	.0064
	12.73		.11	.0121
O	14.37	14.37	0	0
	14.36		.01	.0001
P	11.85	11.85	0	0
	11.85		0	0
Q	14.79	14.73	.06	.0036
	14.70		.03	.0009
	14.70		.03	.0009
R	17.19	17.17	.02	.0004
	17.14		.03	.0009

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 6*

FACTORS USED TO ESTIMATE THE STANDARD DEVIATION FROM RANGE

Size of Sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.591
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

*Natrella, Experimental Statistics(8), pp. 2-6.

Table 7

SAMPLE: Ohio River Water

DETERMINATION: Nitrate (Modified Brucine)

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
0.65 mg/lN	-0.07	.0049
0.68	-0.04	.0016
0.70	-0.02	.0004
0.76	+0.04	.0016
0.79	+0.07	.0049

COMPARATIVE METHODOLOGY AND QUALITY CONTROL

I ROUND ROBIN, COLLABORATIVE OR COOPERATIVE STUDIES ARE UNDERTAKEN FOR A VARIETY OF MOTIVES

A To accumulate data that may be used to determine the precision and accuracy of a new or modified test procedure.

B To recheck an established procedure to ascertain whether there has been a deterioration in the accuracy arising from departures from prescribed routine.

C To test the applicability of an established procedure to new materials.

D To maintain a periodic check on the performance of a group of laboratories.

II DEFINITION OF TERMS

A Series - A number of test results which possess common properties that identify them uniquely.

B Mean - The sum of a series of test results divided by the number in the series. Arithmetic mean is understood.

C Precision Data - Measurements which relate to the variation among the test results themselves - i.e., the scatter or dispersion of a series of test results, without assumption of any prior information. Sometimes referred to as indeterminate error or random error. The following measures apply:

  1 Variance - The sum of squares of deviations of the test results from the mean of the series after division by one less than the total number of test results.

  2 Standard Deviation - The square root of the variance.

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

X_i = value of single results

$\overline{X}$ = mean

n = number of results

  3 Relative Standard Deviation - The standard deviation of a series of test results as a percentage of the mean of this series. This term is preferred over "coefficient of variation."

  4 Range - The difference in magnitude between the highest test result and the lowest test result in a series.

D Accuracy Data - Measurements which relate to the difference between the average test results and the true result when the latter is known or assumed. The following measures apply:

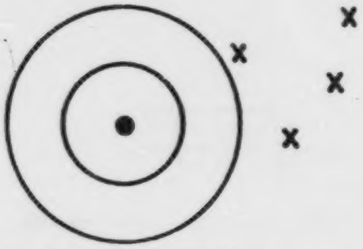
  1 Mean Error - The average difference with regard to sign of the test results from the true results. Also equal to the difference between the average of a series of test results and the true results. Sometimes referred to as determinate error or bias which may result from:

    a Incorrectly calibrated equipment.

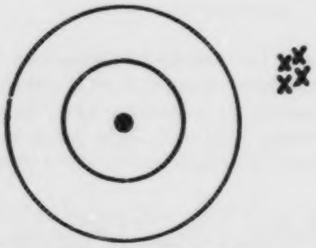
    b Method error.

    c Personal mistakes.

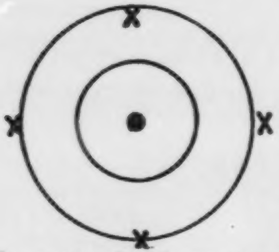
  2 Relative Error - The mean error of a series of test results as a percentage of the true result.



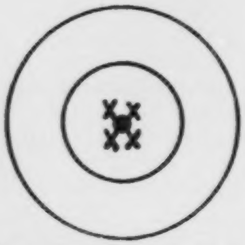
Inaccurate and Imprecise



Inaccurate, but Precise



Accurate, but Imprecise



Accurate and Precise

PRECISION AND ACCURACY

III COLLABORATIVE STUDY OF A METHOD

A The Test Method

1 Identical copies of a detailed description of all steps involved in the test method should be made available to all persons involved in the study.

2 In some cases, it may be desirable to evaluate various portions of the procedure as well as the over-all method. For example, in the analysis of pesticides: (See Attachment 1)

   a The extraction and cleanup step.

   b The determination step.

3 Should a single analysis or duplicate or triplicate analysis be required?

B The Test Sample

1 Necessary samples for the test should be supplied to each collaborator.

2 Generally, three samples providing for three levels of concentration, but concentrations should not be near limits of sensitivity or easily surmised values.

3 It is generally desirable to include both spiked samples and naturally contaminated samples.

4 In some cases, it may be necessary to supply additional samples containing varying but known amounts of interfering materials.

C Collaborating Laboratories

1 An indispensable condition for conducting a successful interlaboratory study is skill and familiarity with the method on the part of all participants.

2 For relatively new methods of test or methods that have been greatly modified, it is advisable to run a pilot study prior to the main interlaboratory test.

D Report Forms

1 The data report sheets should be identical blanks that are designed in an appropriate way for the easy recording of all raw data to be subjected to statistical analysis. (See Attachment 2)

2 Space should be provided for recording of any information pertinent to interpretation of the data.

E Statistical Analysis of Data

1 Usually requires the use of a desk calculator or a high speed computer.

2 Analysis of data should be carried out by person(s) conversant with statistical methods.

3 Interpretation of the results of a study is a joint responsibility of the statistician and a competent analyst who has working familiarity with the method under study. (See Table 1)

IV COMPARATIVE STUDY OF METHODS

A A single sample or series of samples are analyzed by two or more methods (details of methods supplied to each collaborator) and the results are compared statistically

V QUALITY CONTROL

A A round robin that takes in a cross section of typical laboratories goes beyond an evaluation of the procedure.

1 The data that will be collected reflect the merits of the procedure and also reflect the performance of the participating laboratories.

2 Poor results may be caused by deficiencies in the procedure, failures to follow the procedure faithfully, poor technique on the part of the analyst, or poor reagents and standards.

Table 1

Sample A. 0.508 μg of Lindane
Per Liter of Water

n	X_i	$X_i - \overline{X}$	$(X_i - \overline{X})^2$
1	0.228	.212	.044944
2	0.144	.296	.087616
3	0.470	.030	.000900
4	0.425	.015	.000225
5	0.512	.072	.005184
6	0.590	.150	.022500
7	0.258	.182	.033124
8	0.455	.015	.000225
9	0.228	.212	.044944
10	0.485	.045	.002025
11	0.398	.042	.001764
12	0.552	.112	.012544
13	0.568	.128	.016384
14	0.502	.062	.003844
15	0.542	.102	.020808
16	0.645	.205	.042025
17	0.098	.342	.116964
18	0.325	.115	.013225
19	0.600	.160	.025600
20	0.172	.268	.071824
21	1.500	rejected	-------
22	0.370	.070	.004900
23	0.642	.202	.040804
24	0.545	.102	.010404
25	0.605	.165	.027225
26	0.475	.035	.001225
27	0.555	.115	.013225
	11.389		.664452

$\overline{X} = 0.44$ $s = 0.16$ Rel. Std. Dev. 36.6

True Value 0.508   Mean Error 0.068
Rel. Error 13.4

3 Judgment of the procedure will be made on the data remaining after deleting absurd results.

4 By the use of standard samples and the standard addition techniques, each laboratory could carry out its own quality control program. If this were the case, the results would more truly represent solely an evaluation of the procedure.

B Rejection of Results and/or Laboratories

1 Control Charts - If the precision and accuracy of a method is known from previous studies or has been determined by the sponsoring or referee laboratories, then limits of normal variability can be set and the results of any laboratory falling outside of these limits can be rejected. (See Figure 1) Control limits for analysis are frequently set at ±3 standard deviations.

2 Probability Curves - When the results reported by the individual laboratories are plotted on normal probability paper, a straight line should develop, if the data are normally distributed. If the data from a few laboratories do not fall on this straight line, they can be rejected. (See Figure 2)

a The accuracy and precision of the method can then be calculated from the remaining data.

3 Ranking of laboratory results

a Suppose nine laboratories analyzed five different samples.

b Assign the rank of one to the laboratory with the highest results, the rank of nine to the laboratory with the lowest result.

c If a tie exists, say for fifth place, assign the rank of 5.5 to the tied laboratories. If three are tied for fourth place, assign the middle rank of five to all three. This maintains the total of the rank at 45.

d When the laboratories have ranks assigned for all five materials, a score is given each laboratory by adding up its ranks. A score of five is the minimum possible, and a score of 45 the maximum.

e The corresponding five percent probability limits for various combinations of numbers of laboratories and number of materials can be calculated or determined from tables. For 9 laboratories and 5 samples, these limits are 9 and 41.

f Laboratory scores exceeding these critical scores should be rejected. (See Table 2)

4 Testing for Extreme Mean (Table 3)

Briefly, the test involves computing a ratio which compares the distance of the suspected value from its neighbors to the range of all or most all of the observations. Reference of the ratio to a table of critical values for test ratios indicates whether such a large ratio is likely on the basis of chance. If the ratio is greater than or equal to the critical value the probability is small that the suspected outlier is from the sample distribution, hence the outlier is rejected.

REFERENCES

1 ASTM Committee E-11, ASTM Manual for Conducting an Interlaboratory Study of a Test Method. ASTM Special Technical Publication No. 335. American Society for Testing Materials, 1963.

2 Youden, W.J. Ranking Laboratories by Round-Robin Tests. Material Research and Standards 3, 9. 1963.

3 Youden, W.J. The Collaborative Test. J. Assoc. Offic. Agri. Chemists 46, 55. 1963.

4 Horwitz, W. Chemists Guidance Manual. U.S. Department of Health, Education, and Welfare, Food and Drug Administration. August 1964.

5 ASTM Committee D-19, Proposed Procedure for Determination of Precision. Manual on Industrial Water and Waste Water. ASTM Special Technical Publication No. 148-F. American Society for Testing Materials. 1963.

Table 3*

Testing for Extreme Mean

Statistic	No. of obs., k	Critical values						
		$\alpha = .30$	$\alpha = .20$	$\alpha = .10$	$\alpha = .05$	$\alpha = .02$	$\alpha = .01$	$\alpha = .005$
$r_{10} = \frac{X_2 - X_1}{X_k - X_1}$	3	.684	.781	.886	.941	.976	.988	.994
	4	.471	.560	.679	.765	.846	.889	.926
	5	.373	.451	.557	.642	.729	.780	.821
	6	.318	.386	.482	.560	.644	.698	.740
	7	.281	.344	.434	.507	.586	.637	.680
$r_{11} = \frac{X_2 - X_1}{X_{k-1} - X_1}$	8	.318	.385	.479	.554	.631	.683	.725
	9	.288	.352	.441	.512	.587	.635	.677
	10	.265	.325	.409	.477	.551	.597	.639
$r_{21} = \frac{X_3 - X_1}{X_{k-1} - X_1}$	11	.391	.442	.517	.576	.638	.679	.713
	12	.370	.419	.490	.546	.605	.642	.675
	13	.351	.399	.467	.521	.578	.615	.649
$r_{22} = \frac{X_3 - X_1}{X_{k-2} - X_1}$	14	.370	.421	.492	.546	.602	.641	.674
	15	.353	.402	.472	.525	.579	.616	.647
	16	.338	.386	.454	.507	.559	.595	.624
	17	.325	.373	.438	.490	.542	.577	.605
	18	.314	.361	.424	.475	.527	.561	.589
	19	.304	.350	.412	.462	.514	.547	.575
	20	.295	.340	.401	.450	.502	.535	.562
	21	.287	.331	.391	.440	.491	.524	.551
	22	.280	.323	.382	.430	.481	.514	.541
	23	.274	.316	.374	.421	.472	.505	.532
	24	.268	.310	.367	.413	.464	.497	.524
	25	.262	.304	.360	.406	.457	.489	.516

* Data taken from Introduction to Statistical Analysis by Dixon & Massey, p. 412.

6 ASTM Committee E-11, ASTM Manual On Quality Control of Materials. Special Technical Publication 15-C. American Society for Testing Materials, January 1951.

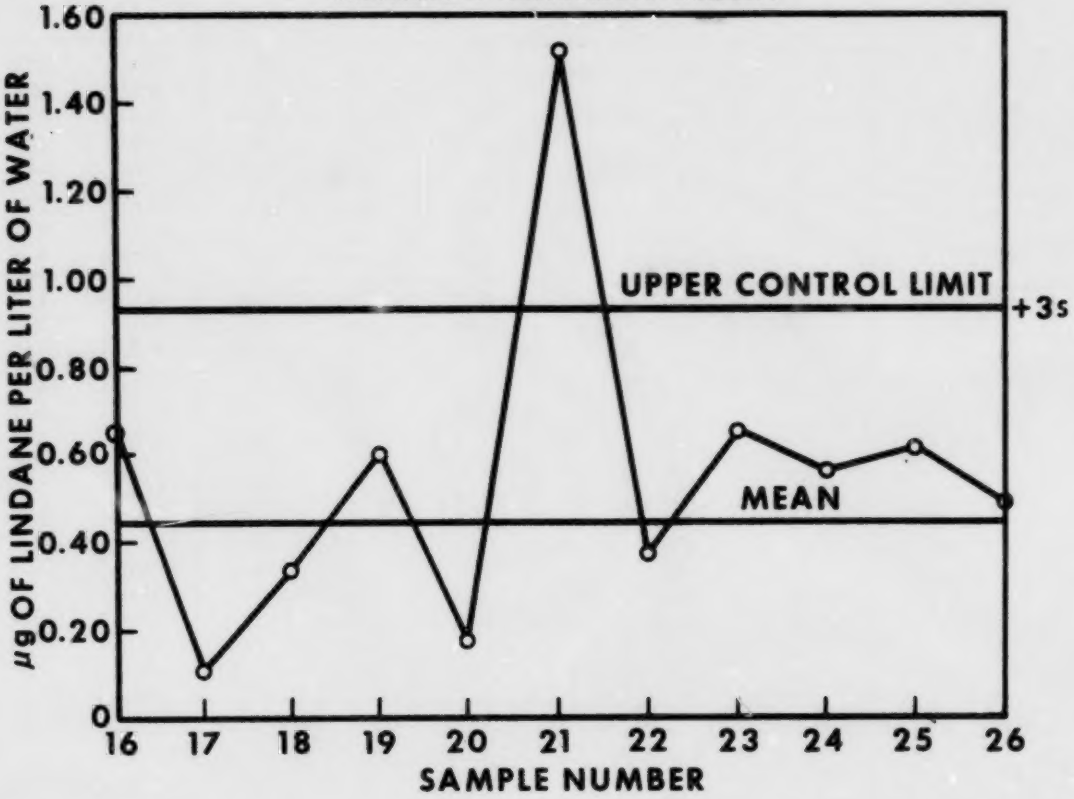
7 Guides for Measures of Precision and Accuracy. Anal. Chem. 34, 364 R. 1962.

8 Accuracy in Clinical Chemistry. Dade Reagents Inc., Miami, Florida.

9 Dixon, W. J. and Massey, F. J. Introduction to Statistical Analysis. p. 276 2nd Edition. McGraw-Hill Book Co., Inc., New York.

This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213

FIGURE 1. CONTROL CHART



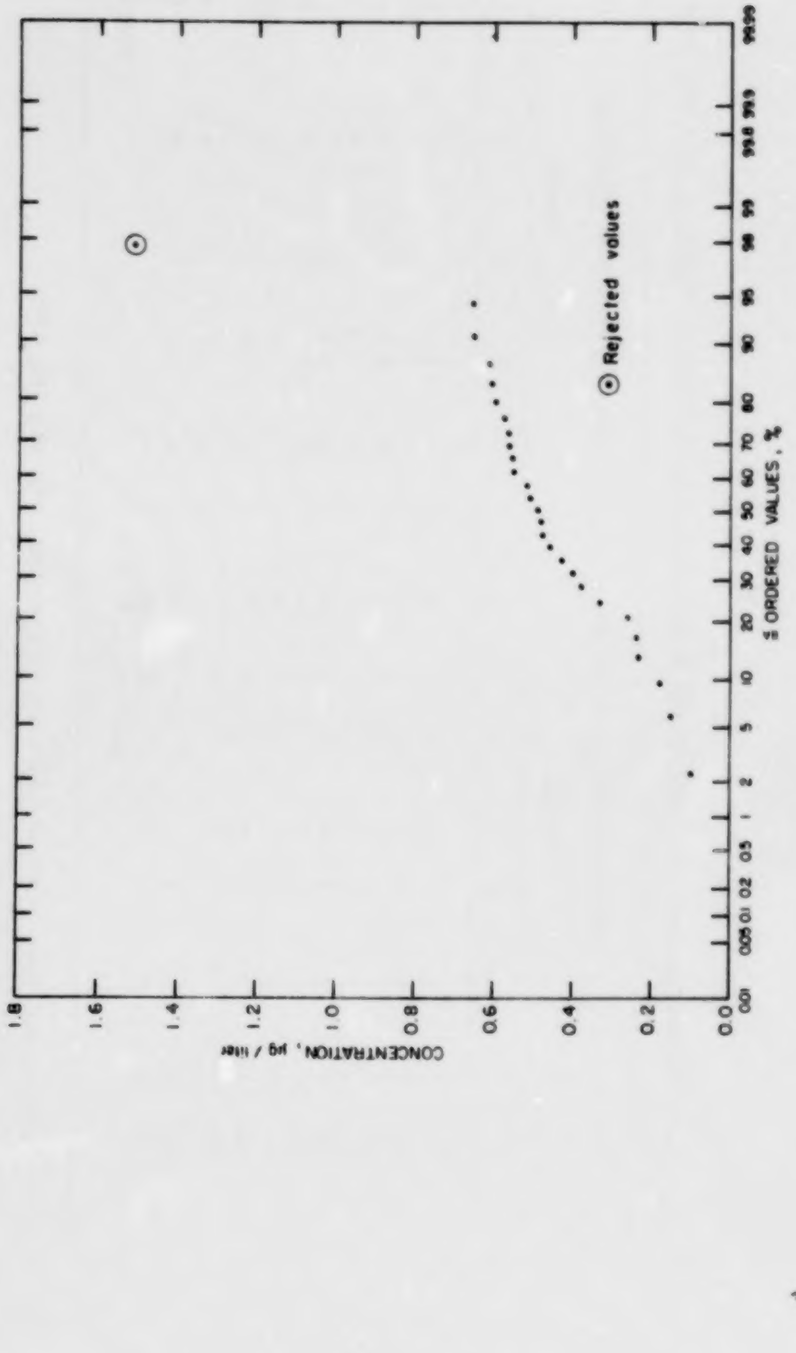


FIGURE 2. PROBABILITY CURVE

Table 2

RANKS AND SCORES OF LABORATORIES REPORTING PERCENTAGE OF TOTAL ALKALOIDS AS NICOTINE[a]

	Collaborator's Choice of Method						Tentative Recommended Method					
	Sample						Sample					
	A	B	C	D	E	Score[b]	A	B	C	D	E	Score[c]
No. 1	...	...	...	...	...	...	6	5	7	10	2.5	30.5
No. 2	1	1	2	2	3	9	2	3	1	6	5	17
No. 3	9	9	7	7	8	40	7	8	4	7	10	36
No. 4	7.5	8	9	6	7	37.5	8	6.5	5.5	8	4	32
No. 5	...	...	...	...	...	...	3	1.5	5.5	3.5	1	14.5
No. 6	6	2	4.5	1	1.5	15	4	6.5	9	1	7.5	28
No. 9	4	4	4.5	4	5	21.5	10	10	10	9	9	48
No. 10	2	3	1	3	1.5	10.5	...	...	...	...	...	...
No. 11	5	6	6	8	5	30	1	1.5	2	2	2.5	9
No. 12	7.5	6	8	9	9	39.5	...	...	...	...	...	...
No. 13a	...	...	...	...	...	...	9	9	8	3.5	7.5	37
No. 13b	...	...	...	...	...	...	5	4	3	5	6	23
No. 15	3	6	3	5	5	22						
				Average....		25.0				Average....		27.5

---

[a] See Journal, Assn. of Official Agricultural Chemists, Vol. 42 p 305, 1959

[b] Critical scores are 9 and 41.

[c] Critical scores are 10 and 45.

AERIAL RECONNAISSANCE IN POLLUTION SURVEILLANCE

I INTRODUCTION

A Definition

The word "reconnaissance" is derived from the word "reconnoiter" which means to conduct a preliminary examination or survey. Its earlier applications to engineering and military requirements has been expanded to include photomapping and interpretations of natural resources. Aerial reconnaissance can be defined as "airborne examination or survey procedures performed by heavier-than-air craft, lighter-than-air craft, or earth orbiting satellites."

B Types of Aerial Reconnaissance

1 Visual

Examination of the flight path by a human observer with no provision for permanent recording for later study. This form of aerial observation has limited use but in many cases can complement the other forms.

2 Image forming sensor

Image recording of the covered flight path where maximum advantage can be made of image interpretation techniques. Image forming sensors includes such instruments as cameras, infrared scanners, and radar.

3 Nonimage forming sensors

Nonimage forming sensors include such devices as oscilloscopes, strip charts, and dial indicators which directly indicate parameter differentials as received by the sensing elements from the target scan.

4 Combination image forming and nonimage sensors

It is advantageous at times to combine both of these techniques in order to rapidly interpret flight path imagery. This advantage is shown in Figure 1 where a heated effluent discharge is traced to a canal by infrared imagery and indicated by the "lighter" plume which parallels the shoreline. The oscilloscope phototracing indicates the temperature differential of the discharge point as compared to the offshore bay area.

C Detected images can be identified by one or more of the following criteria.

1 Size of image

2 Shape of image

3 Tonal qualities of image

4 Image profile shadowing

5 Location of image

6 Texture patterns of grouped images

7 Spatial relationship of image to surrounding bodies

D Selection of Remote Sensing Method

1 A knowledge of the electromagnetic spectrum (Figure 2) is important in order to select the most advantageous method of remote sensing. It will be noted that there are transmissibility and sensitivity limits as well as existing film limits with respect to equipment wave length patterns.

2 Remote sensing methods can be categorized into two areas.

a Source active: Utilization of an instrument which is capable of emitting a source of energy which is transmitted to the target area and emanations are received whose characteristics are dependent upon the nature of the specific target.

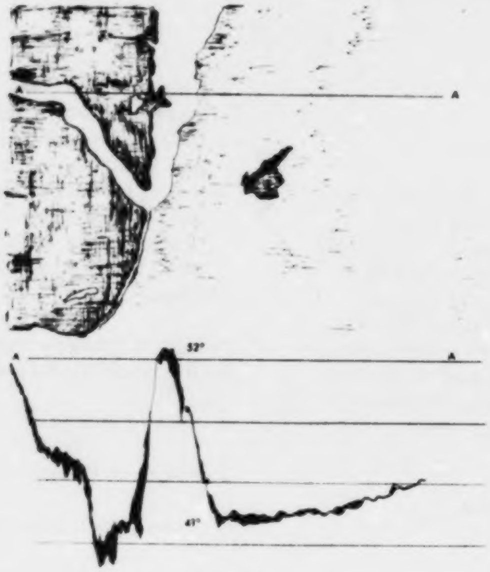


FIGURE 1

An example of such a device is the Radar set (Figure 3) or special Infrared Emitters both of which are capable of being utilized in total darkness.

b Source passive: Utilization of instrumentation which is only capable of receiving target area emanations. Such remote sensing instruments, therefore, do not transmit artificial energy sources but depend upon the sun as a source of energy which is selectively received and individually reflected by each object in the target area. Aerial photography and most of the remote sensing is by this method and the included regions of the electromagnetic spectrum are the upper portions of the UV band to the near infrared region.

3 Selection of desired wavelength is dependent upon the optimum emission capability of the instrument which has the shortest wavelength possible to sharply differentiate small objects while being long enough to preclude excessive energy "scattering" and yet not long enough so that photographic recording is impossible.

II TECHNIQUES

A Radar(RAdio Detection And Ranging)

Radar is particularly useful for discerning certain types of vegetation which may not appear in the best visual quality in Panchromatic Color Film (sensitive to the entire visible spectrum) and also has all-weather and around-the-clock capabilities. Radar in the longer wavelength ranges is capable of penetrating dense vegetative cover and this advantage has been found to be useful in detecting drainage networks and geological features.

B Conventional Photography

Conventional photography covers the entire range of the visible spectrum and employs both black-and-white film and color film. Some of the special films are capable of photographing a portion of the infrared band (the "near" infrared region) and this capability is useful in special situations.

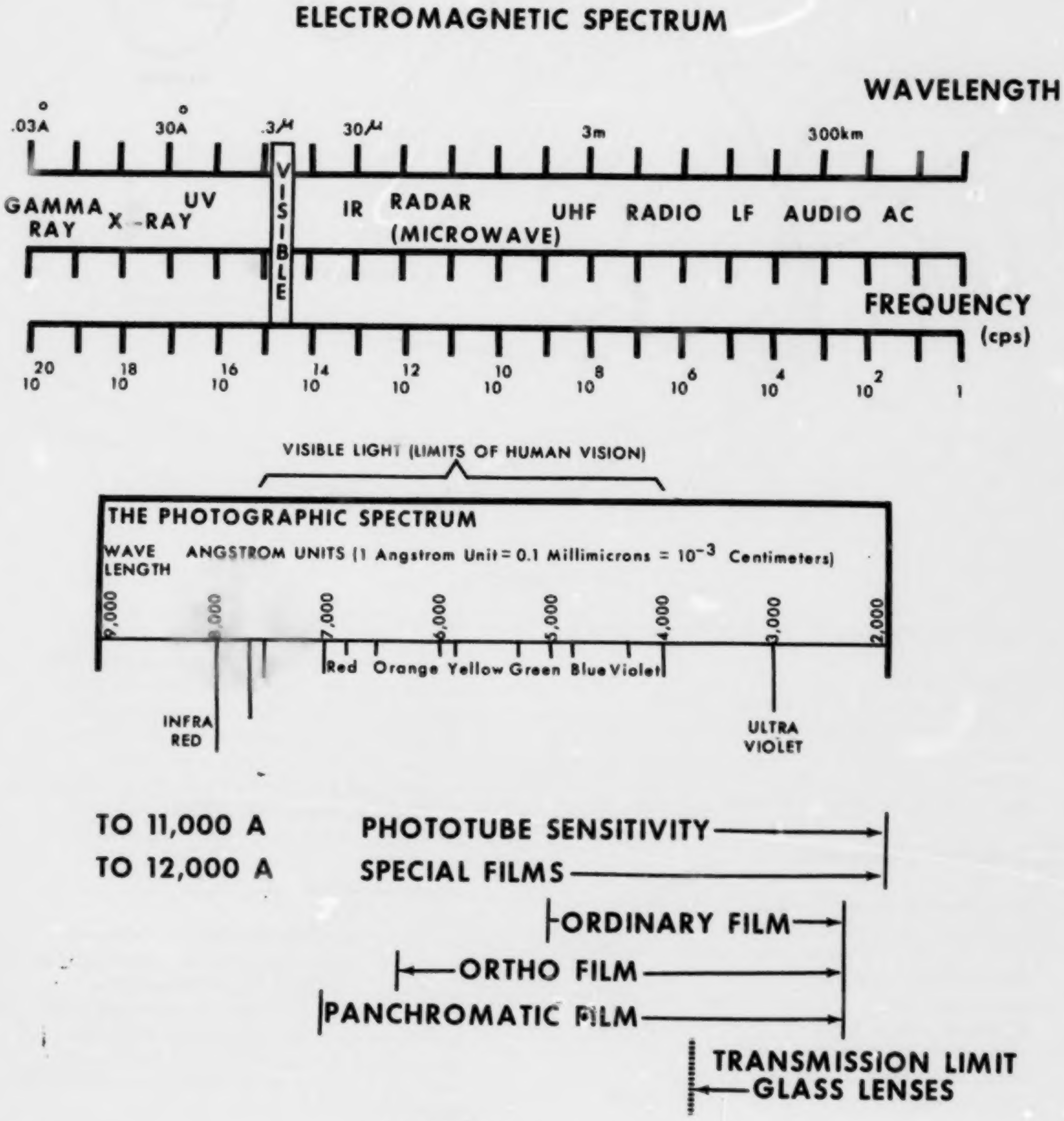

ELECTROMAGNETIC SPECTRUM
WAVELENGTH
.03Å
30Å
.3μ
30μ
3m
300km
GAMMA RAY
X -RAY
UV
VISIBLE
IR
RADAR (MICROWAVE)
UHF
RADIO
LF
AUDIO
AC
FREQUENCY (cps)
10^20
10^18
10^16
10^14
10^12
10^10
10^8
10^6
10^4
10^2
1
VISIBLE LIGHT (LIMITS OF HUMAN VISION)
THE PHOTOGRAPHIC SPECTRUM
WAVE LENGTH
ANGSTROM UNITS (1 Angstrom Unit = 0.1 Millimicrons = 10^-3 Centimeters)
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
Red
Orange
Yellow
Green
Blue
Violet
INFRA RED
ULTRA VIOLET
TO 11,000 A
TO 12,000 A
PHOTOTUBE SENSITIVITY
SPECIAL FILMS
ORDINARY FILM
ORTHO FILM
PANCHROMATIC FILM
TRANSMISSION LIMIT GLASS LENSES

FIGURE 2

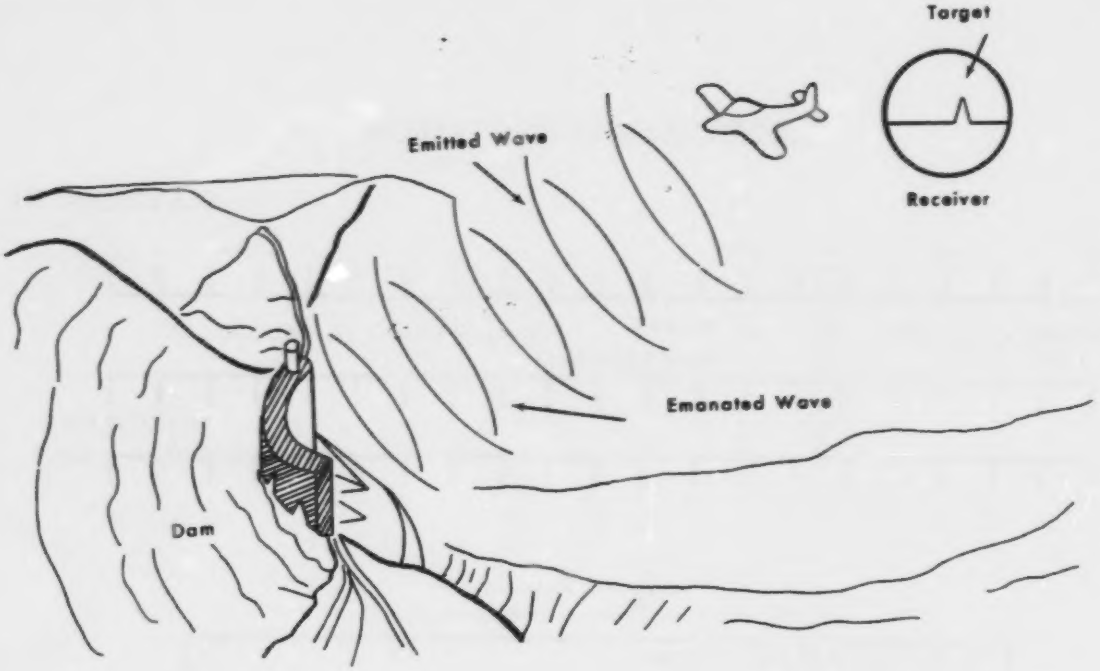


FIGURE 3

1 Direct photomapping and panoramic scan

Figure 4 illustrated the manner in which direct photomapping differs from the panoramic scanning technique and it can be seen that continual attached mapping scans can be made of all or portions of the flight path to be analyzed.

2 Multiband photography

Multiband photography is, as its name implies, the synchronized photography of up to nine separate photographs of the same target area each of which is photographed in a different wavelength. A distinct advantage of this method is that, during photo analysis, distinct anomalies may be evident between objects appearing in the different bands and further investigation may be warranted. An example of this is shown in Figure 5 where it was observed that an anomaly in vegetative growth was apparent in the Infrared wavelength while not being apparent in the other visual spectral ranges. Further investigations proved that gravel washings in slug intervals had affected normal growth of the streamside vegetation.

3 Infrared photography

Infrared photography has been found useful in depicting certain hydrological features. Within the photographic ranges it is not a heat-measuring tool and therefore cannot detect thermal anomalies within bodies of water but instead sharply delineates shoreline and tributary features and has haze penetrating qualities. The blues appear much darker by infrared photography and the reds, greens, and yellows will show up much lighter when compared to a panchromatic color photograph.

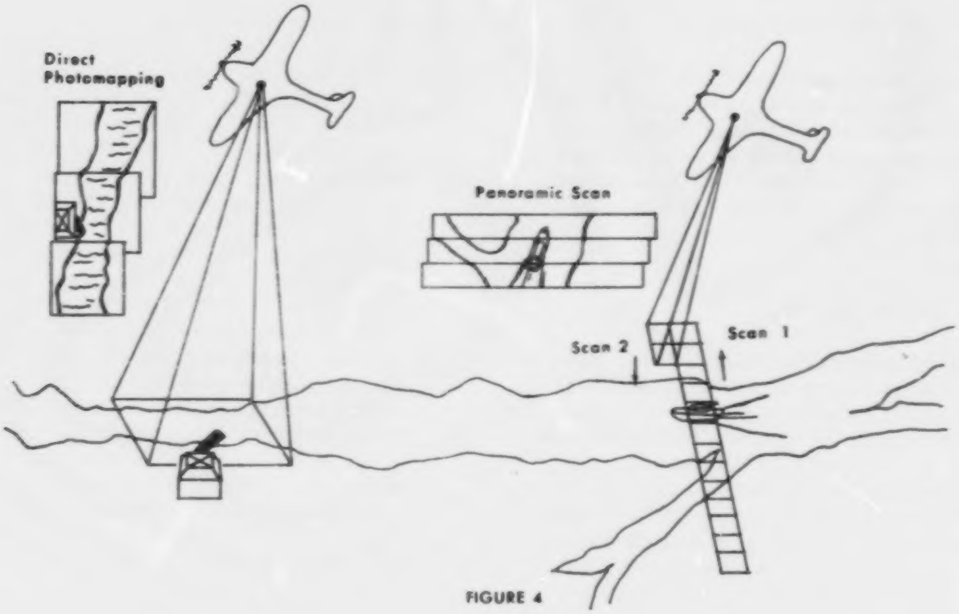

Direct Photomapping
Panoramic Scan
Scan 2
Scan 1

FIGURE 4

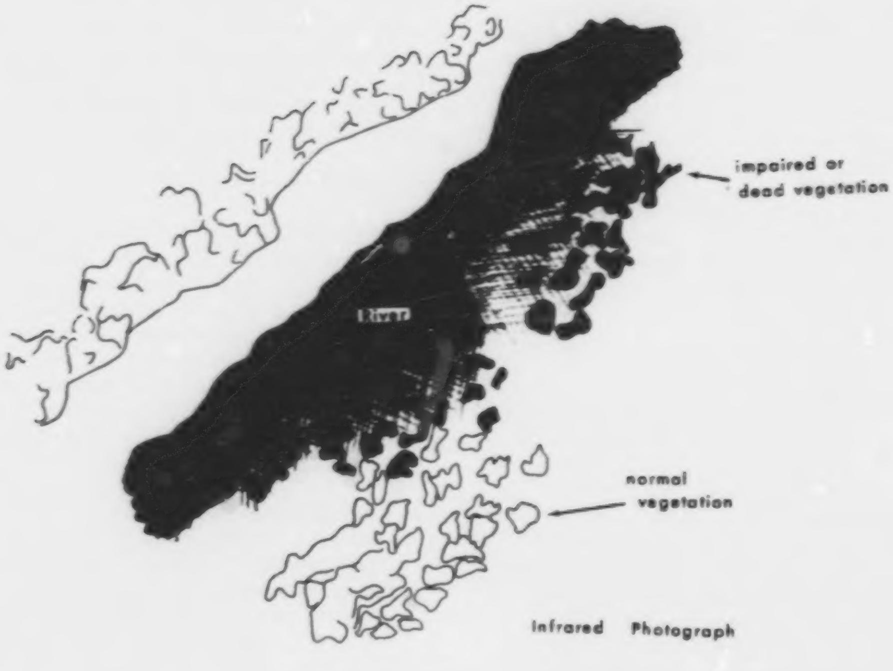

impaired or dead vegetation
River
normal vegetation
Infrared Photograph

FIGURE 5

4 Gamma Ray Spectrometer

This device functions in the very short wavelengths and has been found to have excellent detecting capabilities for radioactivity. Such a device would serve as an excellent means of searching for radioactive waste spills which have occurred either accidentally or intentionally.

C Infrared Radiometry

Infrared imagery is different than infrared photography since the tone of the imagery is directly related to the infrared radiation emitted from the target area. This type of scanner converts the normally unphotographic IR band to a thermal imagery. An optical-mechanical device is necessary since the normal thermal energy emitted by the camera itself will influence radiations received from the target area and this elimination of detector internal radiation is accomplished by miniturization and application of extremely low temperatures. One example of the usefulness of infrared imagery is shown in Figure 6 where the dark areas along the shoreline were found to be caused by cold water infiltration by a previously unknown source. Such a finding could be indicative, for example, of a new untapped freshwater source or a polluting influence from a remote source. This cold water infiltration was not evident by normal visual spectrum photography and the IR photograph would only indicate a more distinct shoreline.

D Automatic Analysis

In the analysis of large numbers of prints use can be made of photoelectric scanners wherein automatic recording of degree of brightness can be recorded on a tape printout. An example of this procedure is shown in Figure 7 where each symbol has a significance relating to "tone signature." The "D" zones are the darker areas while the other symbols relate to lighter zones. In the center the clear area is a river tributary. Future predictions are that automatic encoding can be channeled to computers wherein decisions can be made, for instance, in water management policies.

E Stereoscopic Analysis

The principles and methods developed during World War II for the stereoscopic analysis of aerial photographs has been a continuing and expanding science. Its principles are based upon the fact that the human eyes are normally spaced about 65 millimeters apart and are capable of compositing, via the optic nerve, the two separate and distinct images viewed by each orbit. Aerial stereoscopic analysis depends upon the placing of two photographs precise distances apart with precise overlapping as required by normal human visual acuity. With the aid of special stereoscopic viewing lenses the viewer is able, in most cases, to composite both of the photographs in the same manner as with normal vision and thus a sense of depth can be imparted materially enhancing the analyzing of the terrain.

III APPLICATIONS

Aerial reconnaissance in pollution surveillance can be accomplished in a variety of ways and the method chosen usually is dictated by costs involved and what is desired with regard to immediate or potential information.

A Visual Observations

The simplest and least expensive method is to visually observe the study area and this can be useful in determining sampling sites, locating previously undetermined tributaries, pinpointing visual gross pollutant effluents, etc. To augment these observations it is usually better to have a permanent recording of the survey area in the form of photographic or imagery recordings.

B Photographic Techniques

Photographic evidence, either color, black-and-white, or special film, can be recorded in various attitudes or positions of the aircraft in order to make best use of interpretation techniques such as stereoscopic analysis.

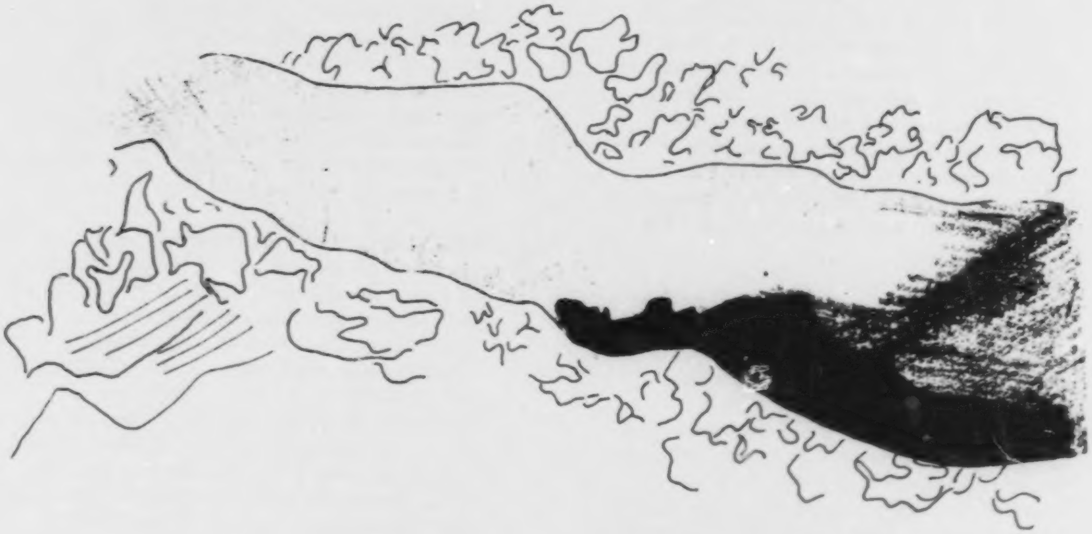


FIGURE 6

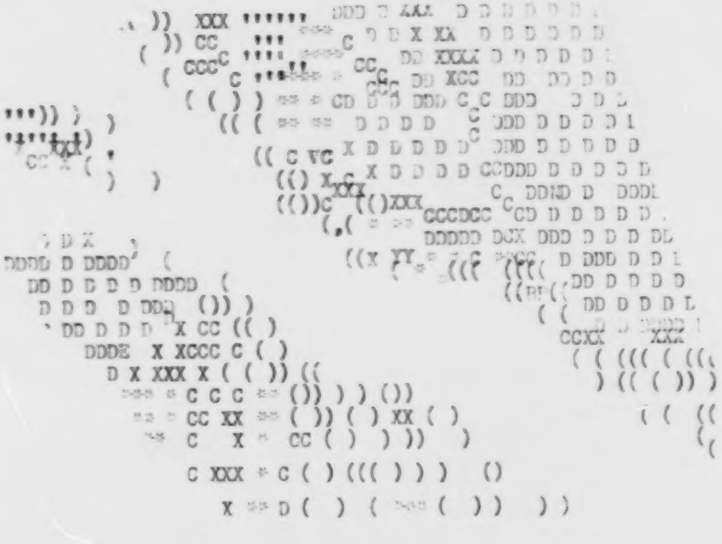


FIGURE 7

1 Current patterns and flow velocity can be ascertained by a method developed by the U.S. Coast and Geodetic Survey whereby powdered aluminum is surface distributed to a wide area and subsequent photographic patterns can be analyzed. Flowing bodies can either appear as "depressions" or "elevations" depending upon direction of flow with relation to the photographing aircraft and from these "parallax" anomalies the velocity can be determined.

2 Photographic IR has found applications in the areas of detecting sharp delineations of shorelines and tributaries especially when haze is prevalent.

3 The work of Strandberg[1] concludes that "In aerial photographs, oxygen-deficient water frequently appears black, or at least darker in tone and this appearance may be caused by the incomplete reduction of wastes by anaerobic bacteria." Thus his black-and-white photographs of waterbodies with low values of dissolved oxygen (DO) following a source of waste discharge are characterized by a plume discharge followed downstream by dark water tones.

Further values ascribed to photography in this manual include color photographs dealing with fish kills where the extent of damage can be better ascessed and the use of "false" color infrared photography where algal masses can be differentiated and, again, the low DO concentration is manifested by a color anomaly.

C Imagery Techniques

1 Utilization of the "mid" and "far" infrared wavelengths in the form of "imagery" has been found useful in analyzing for thermal pollution and its concomitant circulation and diffusion patterns for which "thermographs" can be described for large water bodies.[2] It is also possible to establish naturally occurring patterns of seasonal and diurnal thermal ranges for surface waters.

2 It has been ascertained, in principle, that it is possible to identify pollutants by study of spectral emissivity characteristics for particular wavelengths and this can be of value, for instance, when one is searching for a particular pollutant and the emissivity characteristics for the waterbody can be compared with known patterns for the specific pollutant.[2]

3 Utilization of radar and orbiting satellite platforms can produce imagery of great usefulness. The radar technique can precisely delineate shoreline characteristics without vegetative interferences and thus can thoroughly image drainage basins. Satellite imagery and photography can obtain the same information but on a much broader scale and with great rapidity. Recent lines of thought have postulated that this orbiting scanner can be fitted with computing devices to render decisions upon analysis of its constant data collection.[3] Such decisions can be of immeasurable aid to the water conservationist who may require long range predictions based upon analysis of whole river basins.

IV SUMMARY

Development of aerial reconnaissance in pollution surveillance has proceeded in the areas of technique and interpretation. With regard to the techniques available most of the earlier problems have been overcome to a degree where available material is amenable to analysis. Such early problems as pitch and yaw of the aircraft, cloud cover, imagery instrumentation, etc., has been overcome to a large degree by available engineering skills and the need for improvement due to military needs. In the area of interpretation, however, much has yet to be accomplished to develop this tool as an extension of laboratory analysis which it will augment rather than replace.

REFERENCES

1 Strandberg, C.H. "Aerial Discovery Manual." John Wiley & Sons, Inc., New York.

2 Van Lopik, J.R., Rambie, G.S. and Pressman, A.E. "Pollution Surveillance by Noncontact Infrared Techniques." Jour. Water Poll. Control Fed., 40, 3, 425, March 1968.

3 Colwell, R.N. "Remote Sensing of Natural Resources." Scientific American. January 1968.

This outline was prepared by R. Russomanno, Microbiologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

V METHODOLOGY MANUAL

	Outline Number
General Procedure for Pesticide Analysis	44
General Procedure for Atomic Absorption Analysis	45
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Analysis of Water Sample for Chlorinated Hydrocarbons	47
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	48
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	49
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	50
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	51
Analysis of Fish Tissue for Chlorinated Hydrocarbon	52
Analysis of Raw Water for Silvex	53
The Beckman Process Carbonaceous Analyzer	54
Carbon-Nitrogen-Hydrogen Analyzer	55

GENERAL PROCEDURE FOR PESTICIDE ANALYSIS

The increasing application of pesticides in large-scale agriculture and in forest areas can contribute to the presence of these toxic materials in a water supply. Contamination can occur through drainage from the surrounding terrain, precipitation from the atmosphere, accidental spills of pesticides in the watershed area, or a cross connection on a distribution system.

Experimental investigations have demonstrated that conventional water treatment processes (in the absence of carbon) are capable of coping with DDT but are considerably less effective against most of the other chlorinated hydrocarbons.

I GENERAL DISCUSSION

A Principle

Gas chromatography is the most useful single technique presently available to the residue analyst. It differs from paper and thin-layer chromatography in that a permanent gas such as nitrogen, argon, helium, or hydrogen is used as the mobile phase. The stationary phase is prepared by impregnating an inert solid with a non-volatile organic liquid. A free-flowing powder is formed which is then packed into a glass or metal tube, usually about one quarter inch in diameter and six feet long. The tube is installed in a heated cabinet, and a stream of gas forced through it at a constant rate. A solution containing a mixture of pesticides is injected into the hot column at the end nearest the gas inlet. The pesticides vaporize and are carried through the column by the moving gas. They travel through the column at different rates owing to differences in their partition coefficients between the liquid stationary phase and the mobile gas phase. Consequently, they emerge from the far end of the column at different times, and pass through a detector which automatically measures their concentrations. The response of the detector is fed into a strip chart recorder so that the presence of each compound is registered as a peak and is measured as it emerges from the chromatograph. Several detectors are available which are capable of indicating the presence of pesticides, but the most sensitive of these is the electron capture detector. This detector yields extremely high responses to halogen-containing compounds and some organic phosphates, but is relatively insensitive to many compounds which do not contain a halogen.

B Interference

In addition to some organic phosphates, many oxygenated compounds, particularly those containing carbonyl groups, give high response to electron capture. Furthermore, as with other chromatographic methods, it is not always possible to be certain that the peak in question is not due to another insecticide of similar retention time which has not been previously studied. In such cases, one of the best techniques for positive identification of pesticide residues is by the use of micro-infrared techniques after trapping fractions from the gas chromatographic column. Unfortunately, this is a tedious and exacting process that requires a great deal of skill, knowledge and equipment that is not generally available. Therefore, in lieu of positive identification by infrared, for routine analysis most residue laboratories use some additional technique such as prior fractionation on a florisil or alumina column, or thin-layer chromatography. The most convenient technique, however, is to compare relative retention times on a minimum of two gas chromatographic columns. The tentative identification made on the one column (polar) is then confirmed by rechromatographing the sample on a second, nonpolar column.

C Detection Limit

Preliminary data indicate the detectable concentrations for chlorinated hydrocarbon pesticide using electron capture gas chromatography is of the order of 0.01 ug/l.

II EQUIPMENT

A Separatory Funnels - 2 liter capacity with teflon stopcock and stopper.

B Erlenmeyer Flasks - 500 ml capacity.

C Chromatographic Column - 20 × 400 mm column with coarse fritted plate on bottom and teflon stopcock.

D Conical Centrifuge Tubes - 15 ml capacity.

E Aluminum Tubing - 1/4" O.D. and 0.032" wall thickness.

F Pyrex Glass Wool.

G Gas Chromatograph - Equipped with dual columns and electron capture detector.

H Strip Chart Recorder - 1 mv.

I Syringes - 10 µl.

J Glassware - All glassware used in pesticide analysis should be rinsed successively with chromate cleaning solution, running tap water, distilled water, and finally several times with redistilled solvent (hexane, petroleum ether or ethyl acetate).

III REAGENTS

A Hexane - AR grade and redistilled (Burdick and Jackson Laboratories, Inc. or Mallinckrodt Nanogram Grade).

B Hydrochloric acid, conc.

C Sodium Sulfate - Anhydrous, granular.

D Florisil - Activated at 1200° F and stored at 130° C.

E Petroleum Ether - Redistilled, bp 30-60° C (Burdick and Jackson Labs., Inc. or Mallinckrodt Nanogram Grade).

F Diethyl Ether - redistilled.

G Mixed Ethers - Mix 1 volume diethyl ether with 3 volumes redistilled petroleum ether.

H DC-200 Silicone Fluid - 12,500 centistokes (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

I Anakron ABS - 80/90 mesh (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

J SE-30 silicone gum rubber (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

K QF-1, fluorosilicone (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

L Ethyl Acetate - Redistilled (Burdick and Jackson Laboratories, Inc. or Mallinckrodt Nanogram Grade).

M Nitrogen Gas - Purified grade.

N Molecular Sieve - 5A (Analabs, Inc. 9 Hobson Avenue, Hamden, Connecticut).

O Stock Pesticide Solution - Dissolve 100 mg of the pesticide of interest and dilute with ethyl acetate to 100 ml to form a solution containing 1.00 mg per 1.00 ml. If necessary, prepare mixtures of pesticides at this same concentration.

P Standard Pesticide Solution - Dilute 1.00 ml stock pesticide solution to 100 ml with ethyl acetate: 1.00 ml = 10.0 µg pesticide.

IV EXTRACTION OF PESTICIDES

A Measure exactly one liter of the water sample into a 2-liter separatory funnel equipped with a teflon stopcock. Acidify the sample with 0.5 ml of concentrated hydrochloric acid and extract successively with 100, 50, and 50 ml of redistilled hexane.

TABLE 1

ELECTRON CAPTURE GAS CHROMATOGRAPHY
USING NONPOLAR COLUMN

(10% DC 200 on Anakrom ABS)

Pesticide	Relative Retention Time*	Pesticide	Relative Retention Time*
methyl ester of 2,4-D	0.29	β-chlordane	1.60
benzene hexachloride	0.38, $\underline{0.47}$	butoxy ethyl ester of 2,4-D	1.78
isopropyl ester of 2,4-D	0.41	DDT (Tech.)	1.81, 2.44, $\underline{3.03}$
lindane	0.47	DDE	1.81
isobutyl ester of 2,4-D	0.61	dieldrin	1.83
isopropyl ester of 2,4,5-T	0.66	*o,p'*-TDE	1.86
n-butyl ester of 2,4-D	0.71	isooctyl ester of 2,4-D	2.00, $\underline{2.37}$
ronnel	0.78	endrin	$\underline{2.05}$, 2.29
heptachlor	0.79	ethyl hexyl ester of 2,4-D	2.07
parathion	0.98	Perthane	2.11
Chlorthion	0.99	endosulfan II	2.16
aldrin	1.00	TDE	2.33
Kelthane	1.01	chlorobenzilate	2.43
n-butyl ester of 2,4,5-T	1.08	*o,p'*-DDT	2.44
Suphenone	1.24	isooctyl ester of 2,4,5-T	2.70, 3.10, 3.65
heptachlor epoxide	1.24	carbophenothion	2.83
chlorbenside	1.36	Dilan	2.83, $\underline{3.37}$
TDE olefin	1.42	butoxy ethyl ester of 2,4,5-T	2.85
α-chlordane	1.43	*p,p'*-DDT	3.03
Perthane olefin	1.47	methoxychlor	4.5
propylene glycol butylether ester of 2,4-D	1.51	Strobane	1.07-3.55
		toxaphene	1.18-4.97
endosulfan I	1.58	tetradifon	5.4
ovex	1.58	coumaphos	9.5

* Aldrin reference; retention time approximately 4.25 min. When more than one peak is present, the major peak is underlined.

B Rinse the drained sample container with three 50-ml aliquots of hexane; the first two rinse volumes to serve as the first extraction volume (100 ml) for the sample and the third rinse serving as the second extraction volume.

C Shake the sample only moderately for four minutes because vigorous shaking may cause severe emulsions, particularly in waters of high organic content and/or high turbidity.

D Combine the extracts in a 500-ml erlenmeyer flask and dry by pouring through a two-inch column of anhydrous sodium sulfate contained in a small funnel plugged with cotton.

E Rinse the column three times with approximately 5 ml of hexane and add the rinsings to the extract in a clean, dry 500-ml erlenmeyer.

F Evaporate the extract nearly to dryness over a steam bath using a current of dry air. Do not overheat the residue or leave the erlenmeyer on the steam bath after all the solvent has evaporated.

V FLORISIL COLUMN CLEAN-UP. This step may be unnecessary when working with finished waters. If so, proceed to step V-D.

A Mount a 20 × 400 mm chromatographic column and fill to a depth of 4 inches with activated florisil with topping; top with about 1 inch of anhydrous granular Na_2SO_4. Add 30 to 40 ml petroleum ether to pre-wet adsorbent and when petroleum ether has just sunk in close the teflon stopcock.

B Dissolve the hexane extract residue in 10 ml of redistilled petroleum ether and add it to the florisil column. Open the stopcock, wash the erlenmeyer with two 10-ml portions of redistilled petroleum ether and add the washings to the column. When the level of the washings is near the top of the column packing, elute the sample by passing 200 ml mixed ethers through the column.

C Collect the eluant in a 500-ml erlenmeyer and then evaporate the eluant to about 5 ml on a steam bath with a current of dry air.

D Transfer sample to a 15-ml conical centrifuge tube, using three rinsings of petroleum ether (1 ml each).

E Evaporate the solvent nearly to dryness. Rinse the walls of the tube with (0.5 ml) diethyl ether and again evaporate nearly to dryness.

F Dissolve the residue and make up to 1 ml in hexane.

VI ELECTRON CAPTURE GAS CHROMATOGRAPHY

Even with sophisticated chromatographic equipment embodying selective detectors, successful residue analysis depends primarily on the chromatographic column. The identification of the pesticide residues in a sample extract rests to a great extent on the retention times of pesticide standards. Furthermore, for quantitative analysis the pesticides contained in complex mixtures must be completely resolved from each other. Therefore, at least two different columns are required, and tentative identifications made by comparing retention times on a nonpolar column are confirmed by rechromatographing the sample on a polar column. Relative retention times of a number of pesticides on two such columns are included in Tables 1 and 2. However, each laboratory should compile tables of retention times for their own instruments and columns.

A Treatment of Nonpolar Packing

Dissolve 2 g DC-200 silicone fluid in 50 ml ethyl acetate in a 250-ml beaker. Slowly add 20 g Anakrom ABS, with stirring, to the other ingredients in the beaker; making sure that all the particles are thoroughly wetted. Evaporate the solvent under a stream of nitrogen or clean dry air while stirring the slurry continuously. When the packing is just damp, spread out in a thin layer on the bottom of a shallow tray in order to air dry. Finally, dry in an oven at 100°C.

B Treatment of Polar Packing

Dissolve 0.8 g SE-30 silicone and 1.2 g QF-1 silicone in ethyl acetate and proceed as described above.

C Preparation of Separate Nonpolar and Polar Columns

Pack 1/4" diameter aluminum column, six feet long, by inserting a glass wool plug into one end of the tube and attaching the same end with a piece of rubber tubing to a vacuum pump or line. Insert the other end of the tube into the packing material so that the material is sucked into the tube. Tap or vibrate the tube to assure a more uniform packing, continuing this operation until the column is filled. Then plug the column with glass wool at the other end.

TABLE 2

RELATIVE RETENTION TIMES OF PESTICIDE STANDARDS ON POLAR COLUMN USING ELECTRON CAPTURE DETECTOR

(SE-30 plus QF-1)

Pesticide	Relative Retention Time*
methyl ester of 2,4-D	0.44
benzene hexachloride (Tech.)	0.45, 0.57, 0.68
methyl ester of silvex	0.46
lindane	0.57
methyl ester of 2,4,5-T	0.67
heptachlor	0.81
aldrin	1.00
Kelthane	1.38
heptachlor epoxide	1.50
endosulfan I	1.92
p,p'-DDE	1.98
Perthane	2.13
dieldrin	2.31
o,p'-DDT	2.62
TDE	2.89
carbophenothion	3.04
endosulfan II	3.07
p,p'-DDT	3.54
endrin	4.13, 6.38
methoxychlor	5.44
tetradifon	9.79

* Aldrin reference. When more than one peak is present, the major peak is underlined.

D Conditioning of the Columns

Install the packed columns in the gas chromatograph oven or other suitable oven and turn on the nitrogen carrier gas. If the column is installed in the gas chromatograph oven, do not connect to the detector but rather vent to the atmosphere to prevent buildup of column bleed in the detector system. Adjust the flow of carrier gas to about 50 ml per minute, and raise the column temperature to 250° C for the nonpolar and 225° C for the polar column. Allow the columns to condition at this temperature for a minimum of sixty hours.

E Molecular Sieve

To remove the moisture and other minor impurities from the nitrogen gas, insert a 1/4" O.D. column six feet in length which has been packed with Molecular Sieve 5A, in the line between the gas cylinder and the gas chromatograph. This is particularly important because trace amounts of moisture reduce the sensitivity of the electron capture detector.

F Standard Curves

Prepare standard curves for all pesticides to be determined. Set up the pesticide series in the range of 0.1 to 1.0 ng per 10 µl by diluting 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the applicable standard pesticide solution to 100 ml with ethyl acetate. Inject these standards into the chromatograph under the same conditions used for the samples. Plot ng of pesticide against peak height to obtain a standard curve. Run check standards immediately after each sample in order to detect any changes in detector response.

G Operating Parameters

Operate the nonpolar column at a column temperature of 190° C, injection block at 230° C, and electron capture detector at 200° C. Maintain the flow rate of nitrogen through the column at 120 ml/min. Adjust the detector purge flow of 30 ml/min. in order to obtain optimum detector response; namely, a total flow through the detector of 150 ml/min. Determine optimum operating conditions for the electron capture detector in the individual laboratory by varying the voltage applied to the detector to find a region where sensitivity is good and response is linear. If necessary, condition the column by repeated injection of mixtures as well as individual pesticides in order to obtain relevant retention times and consistent peaks. A typical gas chromatogram obtained with the electron capture detector and the nonpolar column is shown in Figure 1.

H Operate the polar column at a column temperature of 180° C, injection block of 210° C and the detector at 190° C. Regulate the flow rate of nitrogen through the column at 100 ml/min. and the detector purge at 50 ml/min. Determine the optimum voltage and condition the column as was done for the nonpolar column.

I Determination of Extraction Efficiency

Add known amounts of pesticide standard solution to control samples and carry through the extraction and cleanup procedure in order to ascertain the percentage recovered. Apply the resulting correction factor to the final calculation of the quantity of pesticide in an unknown sample.

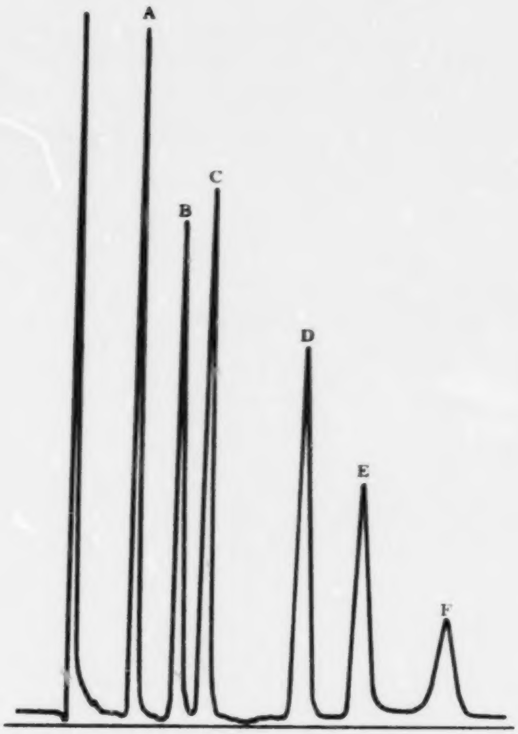

Figure 1. Electron Capture Gas Chromatogram of Pesticide Standards on Nonpolar Column

A. Lindane
B. Heptachlor
C. Aldrin
D. DDE
E. TDE
F. p,p'-DDT

VII CALCULATION

$$\mu g/l \text{ pesticide} = \frac{A \times B \times C}{D \times E \times F}$$

A = ng of pesticide found in solution injected in gas chromatograph.

B = volume of cleanup extract in ml.

C = dilution of extract prior to injection in gas chromatograph.

D = volume in μl injected in gas chromatograph.

E = efficiency of extraction expressed as a decimal.

F = volume of original sample in milliliters.

REFERENCES

1 Breidenbach, A.W., et al. 1964. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. U.S. Department of Interior, Federal Water Pollution Control Administration. Publication No. WP-22. 2nd edition. November 1966.

2 Burchfield, H.P., Johnson, D.C. and Storrs, E.F. Guide to the Analysis of Pesticide Residues. Office of Pesticides, Public Health Service, Washington, D.C. 1965.

3 Burke, J. and Guiffrida, A.L. Investigation of Electron Capture Gas Chromatography for the Analysis of Multiple Chlorinated Pesticide Residues in Vegetables. J. Assoc. Offic. Agr. Chemists. 47. 326. 1964.

4 McCulley, K.A. and McKinley, W.P. Determination of Chlorinated Pesticide Residues in Fat by Electron Capture Gas Chromatography. J. Assoc. Offic. Agr. Chemists. 47. 652. 1963.

5 Teasley, J.I. and Cox, W.J. Determination of Pesticides in Water by Microcoulometric Gas Chromatography after Liquid-Liquid Extraction. J. Amer. Water Works Assoc. 55. 1093. 1963.

This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.

GENERAL PROCEDURE FOR ATOMIC ABSORPTION ANALYSIS

Magnesium, copper, iron, and zinc can be determined by atomic absorption with a sensitivity limit that is lower than the established drinking water standards. Therefore, in evaluating the safety of drinking water, these metals can be determined by simply aspirating the aqueous solution into the flame. Chromium, manganese, and silver can likewise be determined, although the precision and accuracy may not be as good. The determination of cadmium and lead at the concentration set for drinking water may be difficult. However, at these lower concentrations these metals can be determined by complexing with a chelating compound, extracting the chelate into an organic solvent and aspirating the organic phase directly into the flame.

I GENERAL DISCUSSION

A Principle

Atomic absorption resembles emission flame photometry in that a sample is aspirated into a flame and atomized. The major difference rests in the fact that flame photometry measures the amount of light emitted, whereas in atomic absorption spectroscopy a light beam is directed through the flame, into a monochromator, and onto a detector which measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms and in the usual flames the ratio of unexcited to excited atoms at a given moment is very high. Since each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed for each element and hence the method is relatively free of spectral or radiation interferences. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

B Interference

The most troublesome type of interference is usually termed "chemical" and results from the lack of absorption from atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule (in the case of phosphate interference with magnesium) or because the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The interference of phosphate in the magnesium determination can be overcome by the addition of lanthanum. In the determination of manganese, the addition of calcium similarly eliminates the silica interference.

II APPARATUS

A Atomic absorption spectrophotometer

B Autoclave

C Glassware - All glassware, including sample bottles, should be rinsed with 1 + 1 HNO_3 followed by deionized distilled water to avoid contamination errors.

III REAGENTS

A Deionized distilled water - Prepare by passing distilled water through a mixed bed of ion-exchange resins. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.

B Hydrochloric acid, conc.

C Stock metal solution - Prepare as directed in Table 1.

TABLE 1

Metal	Wavelength mμ	Stock solution, page reference[a]	Sensitivity (ppm per 1%)[b]
Zinc	213.8	318	0.04
Chromium	357.9	124	0.15
Cadmium	228.8	69	0.04
Copper	324.7	131	0.20
Magnesium[c]	285.2	171	0.015
Manganese[d]	279.4	174	0.15
Silver	328.1	268	0.10
Lead	283.3	164	0.50
Iron	248.3	157	0.30

[a] SMEWW, 12 Ed.

[b] ppm of metal for 1% absorption in an aqueous solution.

[c] Add lanthanum to overcome phosphate interference.

[d] Add calcium to overcome silica interference.

D Standard Solutions - In general, prepare a series of standard solutions containing 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mg/l by appropriate dilution of the stock solution.

E Fuel and Oxidant - Use an air-acetylene flame for all the metals in Table 1.

F Special reagent for the determination of magnesium - Stock lanthanum solution: Dissolve 58.65 g lanthanum oxide, La_2O_3, in 250 ml of concentrated HCl. Dilute to 1000 ml with distilled water to produce a solution containing 5 g La per 100 ml. Add the acid slowly until the material is dissolved. Add sufficient amounts of this solution to the magnesium standards and unknowns in order to obtain a final working concentration of 1 g La per 100 ml volume.

G Special reagent for the determination of manganese - Stock calcium solution: Dissolve 2.4752 g calcium carbonate in the minimum volume of hydrochloric acid, and dilute to one liter to obtain a solution containing 1000 mg/l calcium. Add sufficient amounts of this solution to manganese standards and unknowns to produce a final working concentration of 50 mg/l.

H Special reagents for the determination of low concentrations of lead and cadmium.

1 Methyl isobutyl ketone

Check for contamination by burning in the flame. If contaminated, wash one volume of methyl isobutyl ketone with five volumes of 1 + 99 hydrochloric acid solution. Remove the excess acid by washing with deionized distilled water.

2 Ammonium pyrrolidine dithiocarbamate solution

Dissolve 4 g in 100 ml distilled water. (K and K Laboratories, Inc., Plainview, N.Y.)

IV PROCEDURE

A Pretreatment of Sample

Acidify all water samples with hydrochloric acid (1 ml concentrated hydrochloric acid per 100 ml sample) and autoclave at 250° F for one hour to solubilize particulate matter.

B Precautions

Install the spectrophotometer on a level bench or table, with the burner under a vent.

C Instrument Operation

Because the differences between the makes and models of satisfactory atomic absorption spectrophotometers render impossible the formulation of detailed instructions applicable to every instrument, follow the manufacturer's operating instructions. As a rule, choose the correct hollow cathode lamp, install, and align in the instrument; position the monochromator at the correct wavelength (Table 1); select the proper monochromator slit width; set the light source current according to the manufacturer's recommendation; light the flame and regulate the flow of fuel and oxidant; adjust the burner for maximum percent absorption and stability; and balance the photometer. Then run the standards which are needed for the working curves and plot the concentrations of the standards against absorbance. If necessary on some instruments, convert percent absorption to absorbance. (The calibration curves are nearly linear for most elements.) Next run the samples and read the concentration of each sample from the calibration curve. For best results run standards each time a sample or series of samples are run.

D Special procedural steps for low concentrations of lead and cadmium

Acidify 200 ml water sample to a pH of 2.2 to 2.8 by adding hydrochloric acid. Add 1 ml ammonium pyrrolidine dithiocarbamate solution and 5 ml methyl isobutyl ketone, and shake for two minutes manually. If an emulsion forms at the interface of the two layers, centrifuge for 10 minutes. Repeat the extraction with a second 5 ml of methyl isobutyl ketone and combine the two extracts. Determine metal in the organic phase against standards which have similarly been extracted. If a precipitate forms in the solvent phase during the first extraction, add an additional 5 ml of methyl isobutyl ketone and repeat the extraction with a second 10 ml. This, of course, will reduce the concentration factor and sensitivity of the method and should not be done unless necessary.

E Aspiration of an organic solvent into the flame

When working with organic solvents the fuel-to-air ratio must be reduced as the burning of the organic solvent contributes to the fuel supply and in addition to lifting the flame off the burner may produce an undesirable luminescent flame. In setting the fuel-to-air ratio of the gas mixture at the burner, start with the settings recommended by the manufacturer for the analysis and gradually reduce the fuel flow (while the solvent is being aspirated) until the flame is as blue as possible and does not lift off the burner. Generally, the fuel flow can be cut almost in half.

V CALCULATION

A For aqueous sample refer directly to the calibration curve.

$$\text{mg/l} = (\text{mg/l in aliquot}) \times D$$

$$D = \text{dilution ratio} = \frac{\text{ml sample} + \text{ml water} + 1\ (\text{ml acid})}{\text{ml sample}}$$

B For extracted sample refer to the extracted standard calibration curve.

$$\text{mg/l} = (\text{mg/l in extract}) \times C$$

$$C = \text{concentration ratio} = \frac{\text{ml solvent}}{\text{ml sample}}$$

REFERENCES

1 Alan, J.E. Spectrochim Acta 17.467. 1961.

2 Willis, J.B. Determination of Calcium and Magnesium in Urine by Atomic Absorption Spectroscopy. Anal. Chem. 33. 556. 1961.

3 Willis, J.B. Determination of Lead and Other Heavy Metals in Urine by Atomic Absorption Spectroscopy. Anal. Chem. 34.614. 1962.

4 Platte, J.A. and Marcy, V.M. A New Tool for the Water Chemist. Ind. Water Engineering. May 1965.

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ATOMIC ABSORPTION LABORATORY

LEAD ANALYSIS

Preparation of Standards:

A stock standard solution containing 1000 mg/l lead was prepared by dissolving 1.5984 g of $Pb(NO_3)_2$ in one liter of distilled deionized water.

From the stock solution, make up 200 ml standards containing 0.025, 0.050, 0.075, and 0.100 mg/l Pb.

Operating Conditions for P-E 303:

Wavelength:	217.
Range:	UV.
Slit:	4.
Source:	10 ma, hollow cathode.
Burner:	P-E premix.
Air:	20 psi. (30 psi. on tank)
Auxiliary Air:	9.0 on flowmeter
Acetylene:	8 psi.; 7.0 on flowmeter.
Flame:	clear and non-luminescent.
Sample Uptake Rate:	2-5 ml/min.

Procedure:

A Extraction of samples and standards:

1 Extract 200 ml aliquots of standards, unknown sample, control sample B, and a blank consisting of distilled water, according to step IV-D of the General Procedure.

B Setting up Instrument:

1 Insert proper hollow cathode tube in instrument.

2 Set operating conditions as specified for wavelength, range, slit, and source current.

3 Turn on the air supply and adjust tank regulator to 40 psi. Set air pressure on Burner Regulator to 20 psi. with auxiliary air valve closed. The air rotameter should indicate about 4.5.

4 Turn on the acetylene supply and adjust tank regulator to 8.0 psi. Adjust the fuel control valve to indicate 4.5 on the fuel rotameter.

5 Ignite the flame.

6 Adjust the auxiliary air needle valve to indicate 9.0 on the air rotameter and the fuel needle valve to indicate 7.0 on the fuel rotameter.

7 Make fine adjustments as required to produce a clear, non-luminescent flame while aspirating the solvent blank.

8 Zero the instrument while aspirating the extracted blank.

C Preparation of Standard Curve:

1 Aspirate the extracted standards and record % absorption.

2 Convert % absorption readings to absorbance by use of Table 1.

3 Plot absorbance readings vs. mg/l Pb.

D Running of Control Samples:

1 Do six determinations on the extracts of control sample A supplied by the instructor. After conversion of % absorption readings to absorbance, determine the concentration of lead in each control sample from the standard curve.

2 Calculate precision and accuracy of the six determinations.

3 Construct a control chart.

E Running of Unknown:

1 Aspirate extracted control sample B and extracted unknown sample and determine if readings are in control using the control chart.

2 If in control, record the concentration of the unknown sample after correcting for any dilution or concentration of the sample. For the calculation, refer to step V of the General Procedure.

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ATOMIC ABSORPTION LABORATORY WORK SHEET

LEAD ANALYSIS

mg/l Pb	% Absorption	Absorbance
0.025	________	________
0.050	________	________
0.075	________	________
0.100	________	________
Control sample A	________	________
	________	________
	________	________
	________	________
	________	________
	________	________

Mean = ______ mg/l Pb   Standard deviation = ____

Control sample B ________

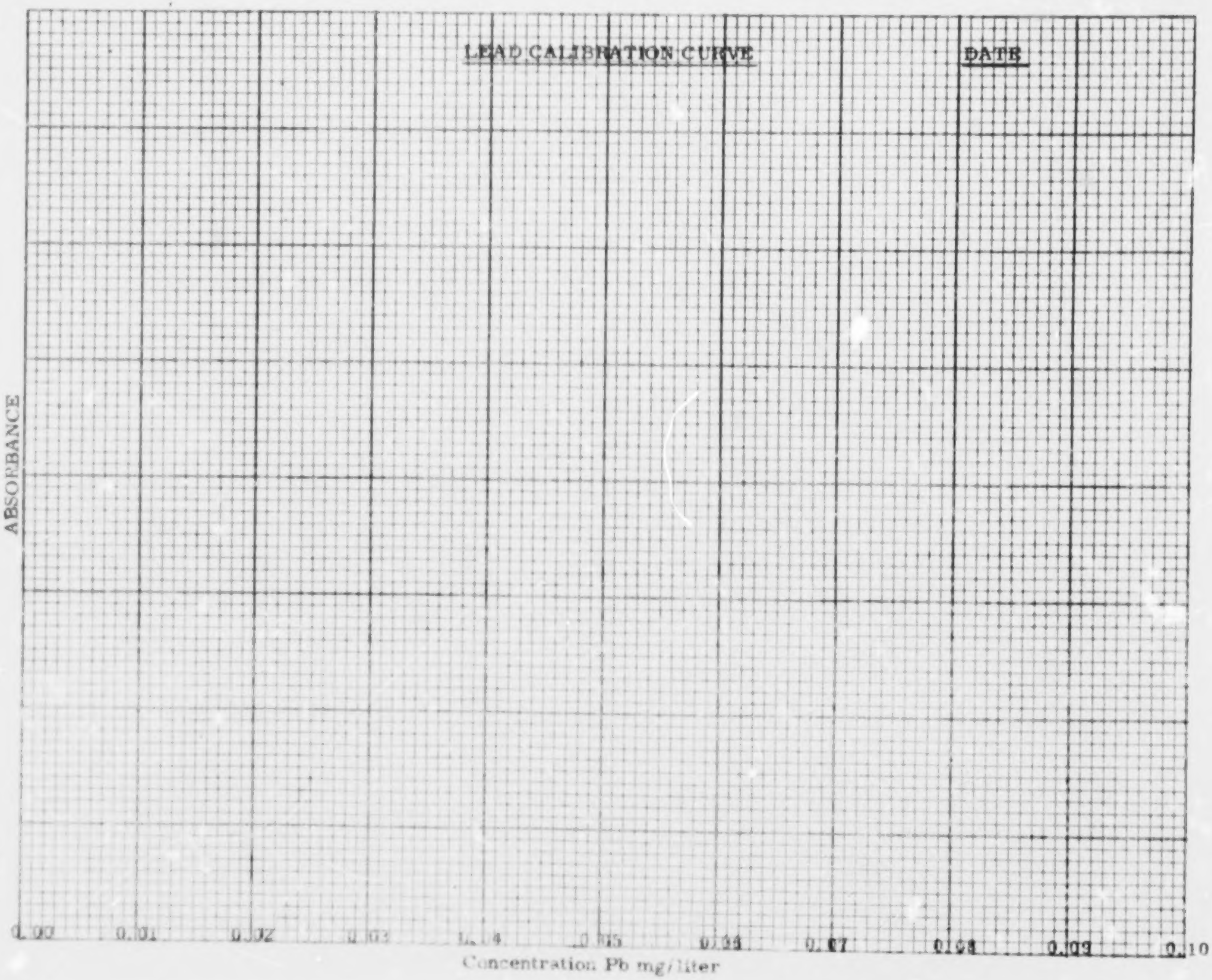
Concentration of control sample B = ________ mg/l Pb

Unknown ________ ________

Concentration of unknown = ______ mg/l Pb

Scale expansion = ________

Suppression (meter response) = ________


LEAD CALIBRATION CURVE
DATE
ABSORBANCE
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
Concentration Pb mg/liter

ANALYSIS OF WATER SAMPLE FOR CHLORINATED HYDROCARBONS

I INTRODUCTION

It is the purpose of this laboratory to acquaint the trainee with techniques for extraction of pesticides from water followed by identification in the Gas Chromatograph.

II EQUIPMENT

A Glass Ware

1 1000 ml separatory flask

2 Kuderna-Danish extractor

3 Funnel

4 Glass wool

5 Medicine dropper

6 Spotting capillary

7 10 ml volumetric flask

8 25 ml graduate flask

B Chemicals

1 Hexane

2 Sodium sulfate

III EXTRACTION PROCEDURE

A Hexane Extraction

1 Add twenty-five ml (25 ml) hexane to 1000 ml separatory flask containing 500 ml water sample. Flask is rocked back and forth gently and shaken lightly for 2 minutes. CAUTION: Release pressure through stopcock.

2 The water sample is drained through the stopcock into a liter beaker. The hexane is poured off the top into an Erlenmeyer flask.

3 The water sample in the beaker is poured back into the separatory flask and another 25 ml portion of hexane is added. Flask is rocked back and forth gently and shaken lightly for 2 minutes. The water sample is drained through the stopcock into a liter beaker. The hexane is poured off the top into an Erlenmeyer flask.

4 Repeat step 3 two more times.

B Drying of Hexane Extract

1 Place a tuft of glass wool in bottom of funnel. Pour granulated sodium sulfate (anhydrous), the Na_2SO_4 filling approximately 2/3 of the funnel volume.

2 Position Kuderna-Danish evaporator on ring stand so that the stem of the funnel containing Na_2SO_4 is well within the neck of the evaporator.

3 Check fitting of collection tube attached to evaporator by two springs.

4 Place a berl saddle in evaporator.

5 Pour the four 25 ml (100 ml vol) hexane extractions through the Na_2SO_4 into the evaporator.

6 Rinse Erlenmeyer flask and Na_2SO_4 several times with approximately 10 ml portions of hexane.

7 Place condenser into the Kuderna-Danish evaporator and take to steam bath.

8 Place evaporator in clamp above steam bath opening.

9 Turn steam up slowly until hexane begins to boil. The berl saddle keeps evaporator from bumping.

10 Evaporation should be adjusted so that the hexane vapors are rushing through condenser.

11 When hexane has evaporated to a few mls in the collector tube, turn off steam and remove the Kuderna-Danish evaporator from the steam bath.

C Preparation of Sample for Gas Chromatography

1 Place a small tuft of glass wool into the tip of a medicine dropper, and fill 2/3 with Na_2SO_4. It is necessary to have an inch of empty space at top of dropper.

2 Place a paper clip on the dropper and set the dropper in the neck of a 10 ml volumetric flask.

3 With another medicine dropper transfer the contents of collector tube into medicine dropper.

4 Using the capillary as a stirring rod pour the last few drops of extract down the capillary into flask. Rinse collector tube and micro Na_2SO_4 column with two dropperfuls of hexane.

5 Bring volume to 10 ml with hexane. Identify volumetric flask with a circle tag.

IV DETERMINATIVE STEP

1 Prepare a standard curve on the electron capture gas chromatograph for lindane, aldrin, and heptachlor epoxide.

2 Inject 5-10 µl of this extract into the electron capture gas chromatograph. Compute the concentrations of the pesticides found in µg/l.

$$\text{NG}\ \frac{\text{pesticide}/10\ \mu\text{l injection}}{1000} \times 1000 \times 2 = \mu\text{g/l pesticide}$$

REFERENCES

1 Breidenbach, A.W., et.al. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Water. U.S. Department of the Interior. Pub. No. WP-22 (1966).

2 Kawahara, F.K., et.al. Semi-Automatic Extraction of Organic Materials from Water. PHS Water Pollution Surveillance Systems Applications and Development Report #16, November 1965.

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I OBJECTIVE

To enable the trainee to identify and quantitate chlorinated hydrocarbon and organophosphorus compounds in a silt loam or red clay sample using soxhlet extraction, florisil column chromatography, thin layer chromatography, anticholinesterase activity measurement and microcoulometric gas chromatography.

II SOXHLET EXTRACTION AND COLUMN CHROMATOGRAPHY

A Reagents

1 Solvents

a Hexane, redistilled in glass BP 68° -69° C

b Petroleum ether, redistilled in glass, BP 30-60° C

c Acetone and ether, ACS grade

d Eluting mixture, 20 parts ethyl ether and 80 parts petroleum ether.

2 Extraction thimbles, Whatman single thickness, 43 x 123 mm.

3 Florisil 60-100 mesh

Florisil is furnished in 3 activation grades: 230° F (110° C), 500° F (260° C), and 1200° F (650° C). Suitably activated product has been heated at 650° C not less than 1 hour nor more than 3 hours. The two lower activation grades must be heated 1 - 3 hours at 650° C in a preheated muffle cooled in an oven at 130° C, transferred to glass-stoppered flasks or bottles and stored in a desiccator. Heat at 130° C not less than 5 hours (overnight is convenient if not used in 10 days). Florisil that has been overheated at 650° C cannot be treated in any way to restore adsorbent properties for this determination.

For further notes on standardization procedures for florisil see reference 1 (Section 2.12).

B Special Equipment Required

1 Soxhlet Extractor, flask capacity (500 ml), with Allihn condenser.

2 Chromatographic tube (20 x 400 mm) with Ultramax valve.

3 Thin layer chromatography equipment.

4 Microcoulometric gas chromatograph equipped with sulfur and chloride cells.

C Preparation of Sample

1 Allow the soil sample to dry thoroughly at room temperature for about a week. This is best accomplished by breaking up the large particles and spreading out in a glass dish.

2 Grind up sample with a mortar and pestle and pass through a #30 or #40 sieve.

3 Select a 100 gram aliquot and place in a pre-extracted thimble. Place a small wad of pre-extracted glass wool on top of the soil.

D Soxhlet Extraction

1 Using 300 ml 10% acetone in hexane, extract soil sample for 24 hours (at 60° C).

2 Transfer extract to 400 ml beaker and concentrate on steam bath (or in Kuderna-Danish evaporator) to about 10 ml.

E Column Chromatography

1 With a stirring rod, insert a small tuft of glass wool into the chromatographic column (to act as a mat for the adsorbent).

To column add 4-1/2 - 5 inches of florisil which has been activated overnight at 130° C and stored in a desiccator to cool. Tamp column to 4 inches using a rubber stopper.

2 Place a 50 ml graduate under column. Add 50 ml petroleum ether to prewet the column. Adjust the elution rate to 5 ml/min (1-2 drops per second).

3 Suspend the extract in 10 ml petroleum ether and place on the column. Rinse the beaker several times with additional small amounts (2-3 ml) of petroleum ether; add these rinsings to the column.

4 Discard the petroleum ether used to pre-wet the column. Place a 200 ml volumetric flask under column and elute with 200 ml 20% ethyl ether in petroleum ether. During elution column may tend to separate. If this occurs, retamp with the rubber stopper. The florisil column does not retain certain pigments present in soil. Consequently, the eluate may have a yellow appearance.

5 After the elution is completed, bring the final volume in the volumetric flask to 200 ml with petroleum ether. Pipette a 10 ml aliquot into a 15 ml centrifuge tube; stopper and label for microcoulometric gas chromatography (MCGC).

F Evaporation of Eluate

1 Transfer remaining eluate (190 ml) to a clean Kuderna-Danish evaporator. Evaporate to 5-10 ml. Transfer to a 15 ml centrifuge tube and evaporate further with clean, filtered air to 0.2 ml.

2 Stopper and label for thin layer chromatography (TLC).

REFERENCES

Barry, H.C., et.al. Pesticide Analytical Manual (Vol. 1). U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, July 1965.

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I THIN-LAYER CHROMATOGRAPHY LABORATORY FOR SEPARATION OF CHLORINATED HYDROCARBON PESTICIDE RESIDUES

A Procedure

1 Preparation of plates

a Weigh a 40 gram aliquot of Al_2O_3 in a 500 ml centrifuge bottle.

b Add 80 ml 0.03 N HNO_3. Shake well and centrifuge at 1200 rpm for 1-2 minutes.

c Decant the supernatant liquid into a 100 ml graduated cylinder, and record the volume (35-40 ml should be recovered).

d Add 80 ml distilled H_2O, break up cake on the bottom of the bottle with a glass rod, shake well and centrifuge as before.

e Decant and record the volume of the supernatant liquid recovered (60-70 ml).

f Repeat the washing step with two additional 80 ml portions of H_2O, centrifuge and decant as before.

g Weigh the Al_2O_3 and H_2O which has been retained (approx. 100 g).

h Add 10 ml 1.0% $AgNO_3$ and enough H_2O to make the total weight 120 g.

i Shake well, place in applicator and prepare plates.

j Let plates dry on mounting board for approximately 15 minutes.

k Place in drying rack in a vertical position at 100°C for 30 minutes.

l Remove the plates and store in a desiccator.

B Separation of Pesticides by Thin-Layer Chromatography

1 Spotting plate

a Position of plate

Scrape 1/4 inch area on two opposite sides of plate. Lay a kimwipe open on top of bench and place an aluminum oxide coated plate on kimwipe.

b Place a kimwipe folded 1/4 size on the left hand side of the work area.

c Place 4 sample vials, unknown, DDT (1μg/μl), DDE (1μg/μl) and dieldrin (1μg/μl) along side of folded kimwipe.

d Insert (5 μl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

e Place template with graduated edge upon the aluminum oxide $AgNO_3$ impregnated plate with starting line about one (1) inch from bottom.

f Mark this position with pencil on both sides of plate. Also mark 10 cm line.

g Rinse capillary twice with DDT removing contents each time on kimwipe.

h At spotting position of template, touch capillary filled with DDT once lightly on plate removing approximately 1/4 of contents of capillary (plate position #1).

i At position #2 spot two times putting 1/2 the contents of the capillary on the plate. Permit each spot to dry before respotting.

j Remove contents of the capillary on kimwipe and refill from vial.

k At position #3 spot the contents of the capillary (5 μl) by lightly touching the capillary to the plate removing about 1/4 of the contents with each spotting. Permit the spots to dry before re-spotting.

l Rinse capillary 5 times in hexane.

m At positions #4 - #6, spot DDE; repeat spotting procedure as described in items h-1.

n At positions #7 - #9, spot dieldrin; repeat spotting procedure as described in items h-1.

o At positions #10 - #12, spot soil extract; repeat spotting procedure as described in items h-1.

2 Development in Solvent

a Place plate, coated surface facing solvent saturated paper, in developing tank containing 1% ethyl ether in hexane with marked spotting line down; develop to 10 cm line.

b Lift off top of jar and remove plate, lifting plate with finger tips of both hands. Dry in air for 10 minutes.

c Place in ultraviolet light box and expose for 15 minutes.

d Measure the distance of centers of the spots from starting line and compute the R_f values. Record data on data sheet.

$$R_f \text{ value} = \frac{\text{distance in cms of pesticide from start}}{\text{distance in cms of solvent front from start}}$$

REFERENCES

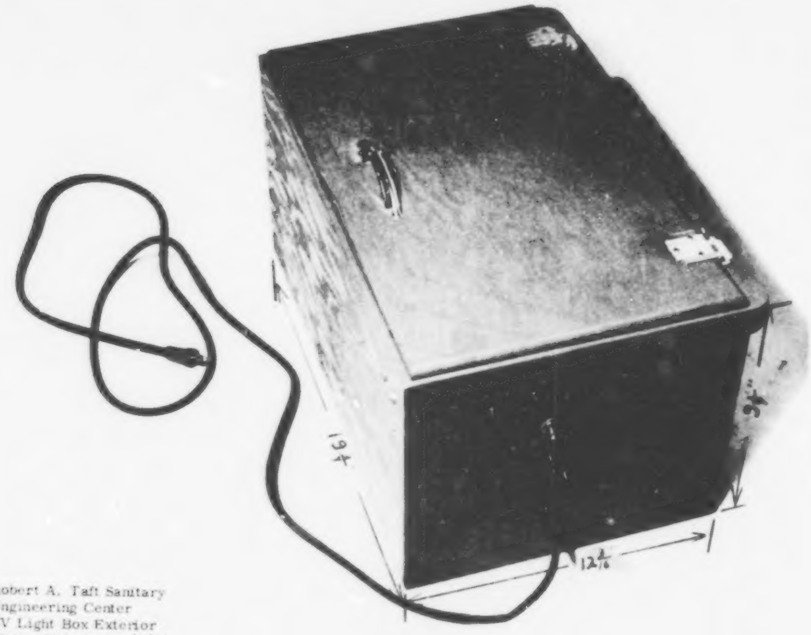
1 Barry, H. C., et. al. Pesticide Analytical Manual (Vol. I). U.S. Department of Health, Education and Welfare, Food and Drug Administration, July 1965.

2 Breidenbach, A.W. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. U.S. Department of the Interior, Publication No. WP-22 (1966).

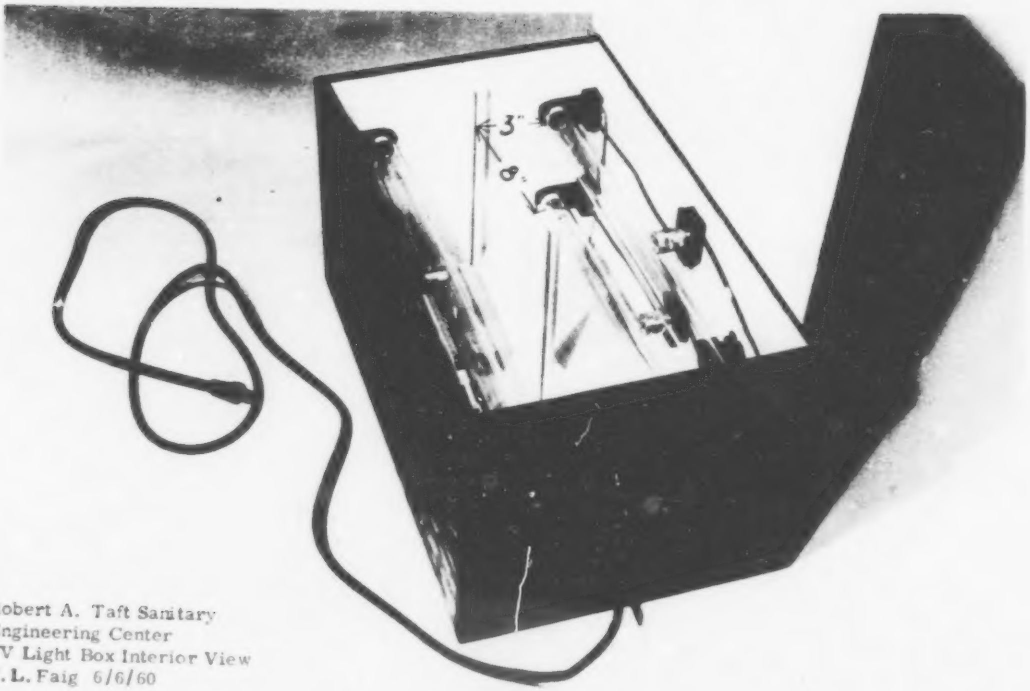
THIN-LAYER CHROMATOGRAPHY DATA SHEET

Sample	Solvent	Solvent Front Distance From Start (cm)	Sample Position Distance From Start (cm)	R_f	R_x

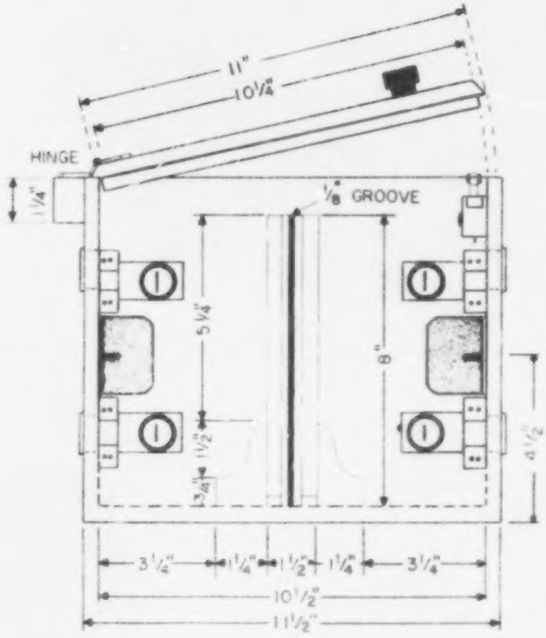
49.3



Robert A. Taft Sanitary
Engineering Center
UV Light Box Exterior
View - H.L. Faig 6/6/60



Robert A. Taft Sanitary
Engineering Center
UV Light Box Interior View
H. L. Faig 6/6/60



SECTION A—A

NOTE:
SEE FOLLOWING SUPPLEMENTAL
SHEET NO.1 FOR CUTWAY VIEW

LEGEND

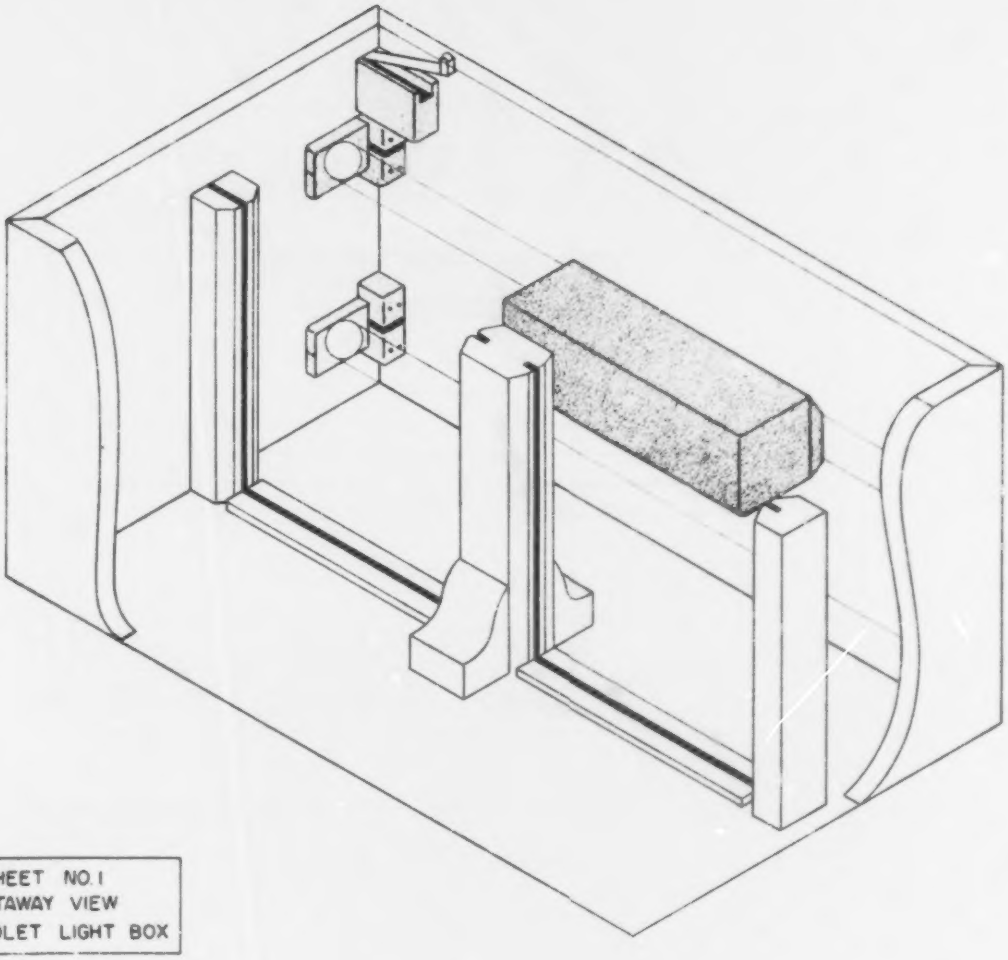
NO. NEEDED	ITEM
4 EACH	FLUORESENT LAMPHOLDER BYRANT #4300 OR EQUIV.
4 EACH	GERMICIDAL LAMPS, 15 WATT GE #G15T8 OR EQUIV.
1 EACH	TOGGLE SWITCH, SINGLE POLE, 10 AMP.
1 EACH	MICRO SWITCH, TYPE W, 10-15 AMP.
2 EACH	BALLAST, TRIGGER START, GE #6G3512 OR EQUIV.
4 EACH	1" ALUMINUM VENTILATING LOUVERS.
1 PAIR	SMALL CABINET HINGES
1 EACH	CABINET PULL
1 EACH	6 FT. LINE CORD

ULTRAVIOLET LIGHT BOX

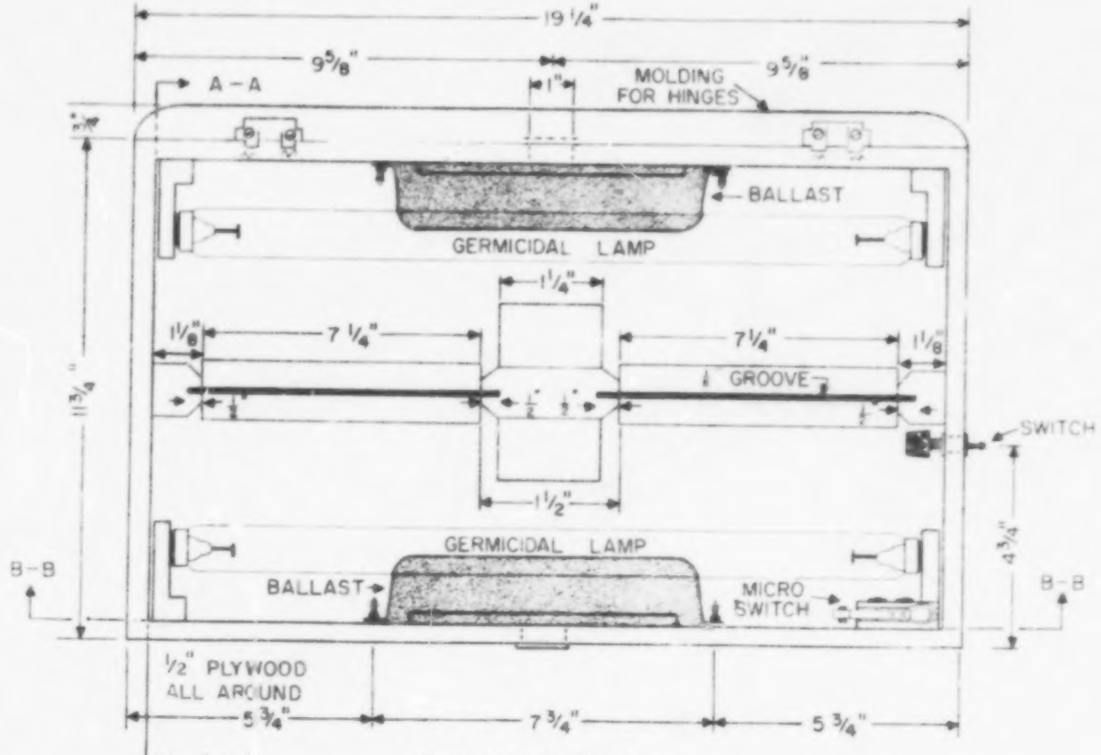
DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
U.S. Public Health Service
ROBERT A. TAFT
SANITARY ENGINEERING CENTER

DESIGNED BY H. L. Falg
DRAWN BY J.C. Boyer from original pilot model
Pilot model constructed in ROBERT A. TAFT SANITARY ENGINEERING CENTER model shop supervised by J. Cortell

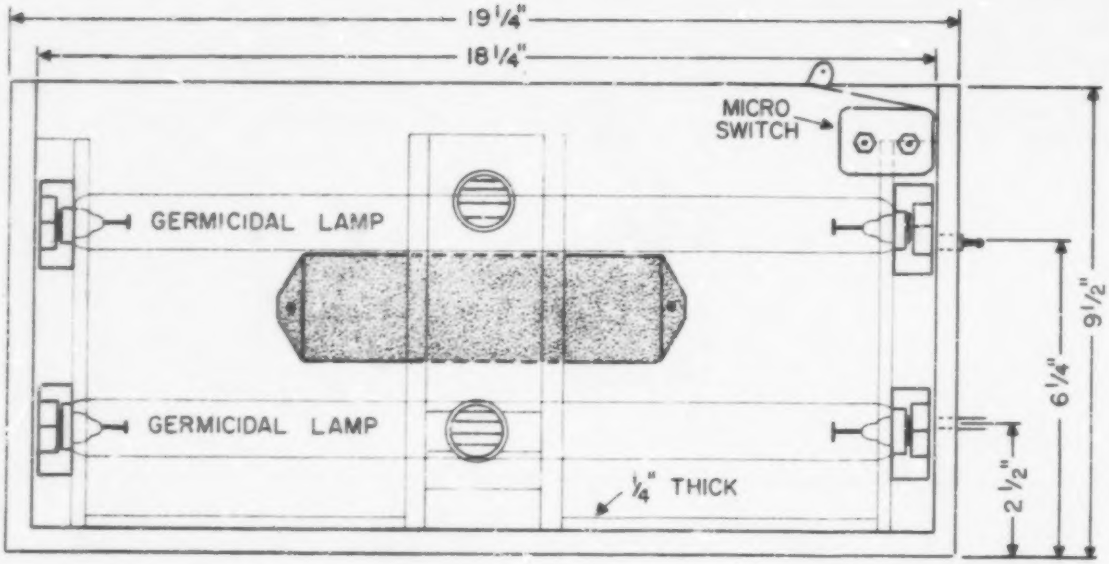
SCALE Date Jan 9, [illegible]



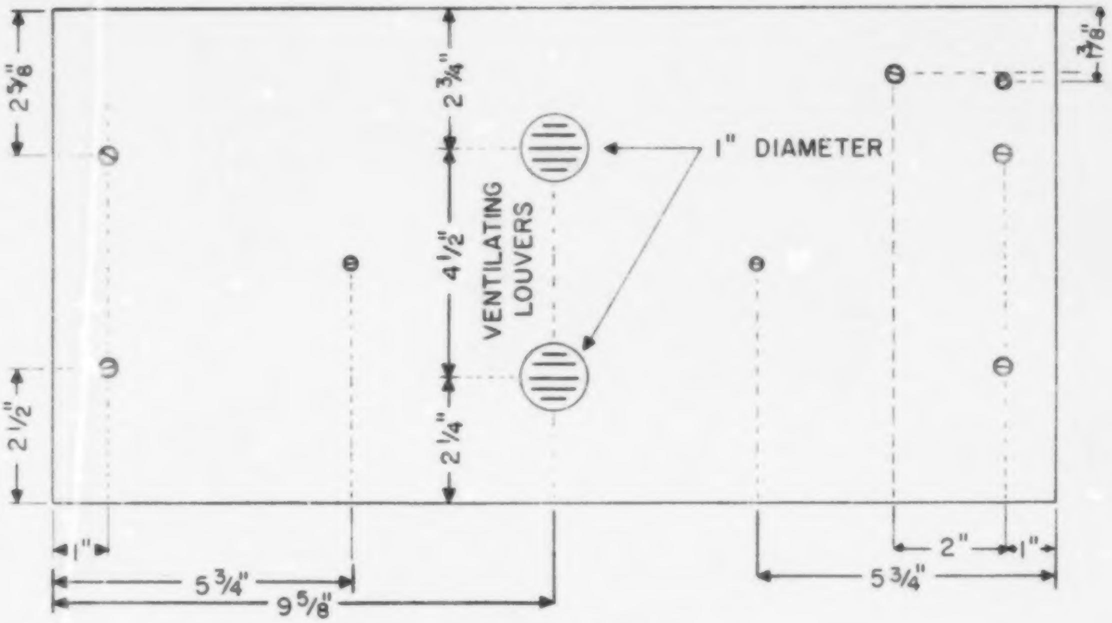
SHEET NO. I
CUTAWAY VIEW
ULTRAVIOLET LIGHT BOX


19 1/4"
9 5/8"
9 5/8"
1"
A – A
MOLDING FOR HINGES
1/2"
BALLAST
GERMICIDAL LAMP
1 1/4"
1 1/8"
7 1/4"
7 1/4"
1 1/8"
1/4 GROOVE
1/2
1/2
1/2
1/2
11 3/4"
SWITCH
1 1/2"
4 3/4"
GERMICIDAL LAMP
B – B
BALLAST
MICRO SWITCH
B – B
1/2" PLYWOOD ALL AROUND
5 3/4"
7 3/4"
5 3/4"
A – A

PLAN (LID REMOVED)


19 1/4"
18 1/4"
MICRO SWITCH
GERMICIDAL LAMP
GERMICIDAL LAMP
1/4" THICK
9 1/2"
6 1/4"
2 1/2"

SECTION B — B


2 7/8"
2 3/4"
3/8"
1" DIAMETER
4 1/2"
VENTILATING LOUVERS
2 1/2"
2 1/4"
1"
5 3/4"
9 5/8"
2"
1"
5 3/4"

FRONT ELEVATION

47-9

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I A MODIFIED KOVAC'S THIN-LAYER CHROMATOGRAPHY FOR ORGANO THIOPHOSPHATE PESTICIDES

A Reagents

1 Adsorbents

Aluminum oxide G

2 Solvent systems

a Immobile

15% - 20% N, N-dimethyl formamide in ethyl ether

b Mobile

Methyl-cyclohexane

3 Chromogenic agents

a Stock dye solution

1 g tetrabromophenolphthalein ethyl ester in 100 ml acetone

b Working dye solution

Dilute 10 ml stock dye solution in 50 ml acetone.

c $AgNO_3$ solution

Dissolve 0.5 g $AgNO_3$ in 25 ml distilled water and dilute to 100 ml with acetone.

d Citric acid solution

Dissolve 5 g citric acid in 50 ml distilled water, dilute to 100 ml with acetone.

B Pesticide Samples

1 1 μg/μl solutions of following pesticides in n-hexane: ronnel, parathion, systox, dipterex, malathion.

2 Combination of two pesticides in one sample 0.5 μg/μl of each: ronnel, parathion.

3 Extract of mud sample containing parathion.

C Equipment

1 Glass plates

8 x 8" double strength coated with 250μ thickness of aluminum oxide G.

2 2 μl capillary and spotting tube

3 50 ml beaker containing n-hexane

4 Developing tank

5 Spray flask or canister

a Tetrabromophenolphthalein ethyl ester dye solution

b $AgNO_3$ solution

c Citric acid solution

d N, N dimethyl formamide in ethyl ether

D Procedure

1 Spotting plate

a Position plate

Scrape 1/4 inch area on two opposite sides of plate. Lay a kimwipe open on top of bench and place an aluminum oxide coated plate on kimwipe.

b Place a folded kimwipe on the left hand side of plate.

c Place 6 sample vials alongside of folded kimwipe.

1) Ronnel

2) Parathion

3) Systox

4) Dipterex

5) Malathion

6) Combination sample

d Insert (2 µl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

e Place template with graduated edge upon the plate with starting line about one (1) inch from bottom.

f Mark this position as spotting line and 10 cm above this line mark the coated surface at the side of the plate.

g Rinse capillary twice with the ronnel solution.

h At spotting position #1 of template, touch capillary filled with ronnel once lightly on plate removing approximately 1/4 of contents of capillary. Permit spot to dry then continue the spotting technique until all of the 2 µl have been applied at the same spot.

i Similarly spot the other pesticides parathion, systox, dipterex, malathion, the combination sample and the soil extract at the next six (6) positions indicated by the graduations on the template. Place 15 ml centrifuge tube containing soil extract aside and mark for determination of anti-cholinesterase activity.

j Rinse capillary 5 times in hexane between each pesticide spotting.

2 Development in Solvent

a Place plate in hood against spraying board with coated surface facing toward front of hood.

b Spray moderately with N, N dimethyl formamide-ethyl ether solution.

c Place plate spotting line down and coated surface facing solvent saturated paper, in developing tank containing methyl-cyclohexane.

d Develop to the mark, and remove plate to hood and place against spraying board.

3 Chromogenic Development

a Immediately spray with tetrabromophenolphthalein ethyl ester solution.

b Spray with $AgNO_3$ solution and let stand for 2 minutes.

c Spray with citric acid solution.

d Measure the distance of centers of the spots to starting line and compute the R_f values.

$$R_f = \frac{\text{distance in cms of pesticide from start}}{\text{distance in cms of solvent front from start}}$$

REFERENCES

Kovacs, M.F. Thin-Layer Chromatography for Organo Thiophosphate Pesticide Residue Determination, JAOAC, Vol. 47, No. 6, 1097 (1964).

THIN-LAYER CHROMATOGRAPHY DATA SHEET

Sample	Solvent	Solvent Front Distance From Start (cm)	Sample Position Distance From Start (cm)	R_f	R_x

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I MICROCOULOMETRIC GAS CHROMATOGRAPHY

A Description of Instrumentation

1 Detector

Dohrmann Model C-200 microcoulometer equipped with T-300 titration cells.

2 Operating conditions

a Column conditions

1) Temperature ______ °C

2) Flow rate ______ ml/min

3) Diameter ______ inches

4) Length ______ feet

5) Solid support ______

6) Stationary phase ______

b Combustion furnace

1) Furnace temperature ______ °C

2) Inlet temperature ______ °C

3) O_2 flow rate ______ cc/min

4) N_2 sweep rate ______ cc/min

c Titration cell

1) Type ______

2) Mode ______

3) Range Ohms ______

4) Bias Volts ______

5) Electrolyte ______

B Background Theory

1 Coulometric titration

a Chloride cell

The microcoulometric titration cell (chloride) used as a gas chromatographic detector has for its basis the coulometric titration of chloride ions with silver ions. Chloride ions are produced when the effluent from the gas chromatographic column enters the combustion furnace and any chlorinated hydrocarbons present are broken down into HCl, CO_2 and H_2O.

1) When chloride ions enter the titration cell, the deficiency of silver ions is registered by a reference/sensor electrode pair (see Figure 1).

2) The voltage difference between the reference/sensor pair and an applied bias voltage is amplified and fed as an input to the generator circuit.

3) A subsequent regeneration of silver ions occurs from the generator anode-cathode pair.

4) During the course of regeneration of titrant, the generation current passes through a series of resistors.

5) The voltage drop across these resistors is fed as the input to the recorder. The area of the peak produced is a measure of the coulombs consumed in the regeneration of titrant, which in turn is a measure of the chloride entering the cell.

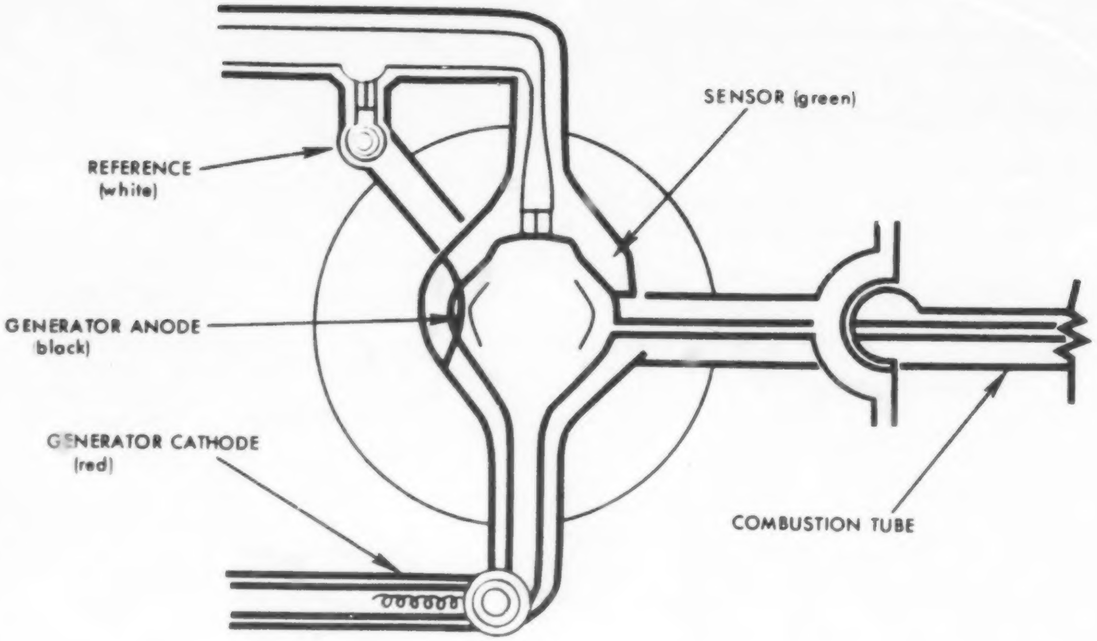


FIGURE 1.

MICROCOULOMETRIC TITRATION CELL

(LOW GAIN MODE)

b Sulfur cell

This cell operates on the same principles as the chloride cell except that sulfur containing compounds produce SO_2 and H_2O in the combustion furnace. The SO_2 produced is coulometrically titrated with tri-iodide ion in the titration cell.

C Chloride containing Compounds (Chloride Cell)

1 Set electrodes in low gain position.

2 Set GAIN switch to LO (This supplies a gain of approximately 200 to the coulometer amplifier).

3 Set FUNCTION switch to BIAS READ and adjust BIAS MILLIVOLTS with BIAS SET knob to 12 divisions to the left of zero. This supplies 240 bias millivolts to the cell.

4 Adjust stirring rate in titration cell to slowest position.

5 Set FUNCTION switch to GENERATOR READ. Generator volt meter should read 0 or to the left of 0. If the meter reads to the right of 0, add fresh electrolyte to the cell and flush through both stopcocks with electrolyte (70% acetic acid) until the meter reads zero or to the left of zero. (NOTE: Final level of electrolyte should be at black mark on cell).

6 Turn recorder power and chart on and adjust needle on recorder to zero with RECORDER ZERO knob on coulometer amplifier (NOTE: Needle on recorder is at 0 when integrator draws a straight line).

7 Set FUNCTION switch to OPERATE. Set RANGE OHMS to 150 (150 ohms) and wait until cell generates enough titrant to reach null.

8 With VENT open, inject 50 NG of dieldrin into the gas chromatograph. Depress MARK button.

9 After one minute close the vent and wait for dieldrin peak. Measure and record the peak height and peak area.

10 With VENT open inject 50 µl of sample eluate from florisil column. After one minute close the vent and wait for peak. Measure peak height and peak area and calculate µg/gm of dieldrin in soil sample.

D Sulfur Containing Compounds (Sulfur Cell)

1 Set electrodes in low gain position.

2 Set GAIN switch to LO. (This supplies a gain of approximately 200 to the coulometer amplifier).

3 Set FUNCTION switch to BIAS READ and adjust BIAS MILLIVOLTS with BIAS SET knob to 6 divisions to the left of zero. This supplies 720 bias millivolts to the cell.

4 Adjust stirring rate in titration cell to slowest position.

5 Set FUNCTION switch to GENERATOR READ. Generator volt meter should read 0 or to the left of 0. If the meter reads to the right of 0, add fresh electrolyte to the cell and flush through both stopcocks with electrolyte (0.05% KI and 0.04% HOAC) until the meter reads zero or to the left of zero. (NOTE: Final level of electrolyte should be at black mark on cell).

6 Turn recorder power and chart on and adjust needle on recorder to zero with RECORDER ZERO knob on coulometer amplifier (NOTE: Needle on recorder is at 0 when integrator draws a straight line).

7 Set FUNCTION switch to OPERATE. Set RANGE OHMS to 100 (100 ohms) and wait until cell generates enough titrant to reach null.

8 With VENT open, inject 500 NG of malathion and parathion. Depress MARK button.

9 After one minute close the vent and wait for peaks. Measure and record the peak heights and peak areas.

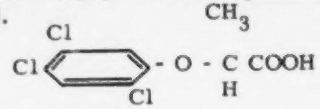
10 Concentrate 10 ml of sample eluate from florisil column to 1 ml in 15 ml centrifuge tube. With VENT open, inject 50 µl of concentrated sample eluate. After one minute, close the vent and wait for peaks. Measure peak height and peak area and calculate µg/gm of pesticide in soil sample.

ANALYSIS OF RAW WATER FOR SILVEX

(Rapid Screening Method)

I OBJECTIVE

To enable the trainee to apply to a raw water sample the technique of esterification in the analysis of the phenylalkanoic acid herbicide silvex 2-(2, 4, 5-trichlorophenoxy) propionic acid.



II REAGENTS

A 1:1 sulfuric acid

B Diethyl ether, anhydrous, reagent grade

C Sodium sulfate (checked for interfering ECGC peaks)

D Boron trifluoride - methanol solution (14% W/V) Available already prepared from Applied Science Laboratories.

E Hexane, redistilled in glass BP 68-69° C.

F Silvex standard (1 ml = 1 µg Silvex in ethyl acetate)

III EXTRACTION

A Pipette 0.2, 0.5, and 1 ml of silvex standard (1 mg/l) into a series of 10 ml volumetric flasks. Evaporate with air and proceed with methylation.

B Add 25 ml of raw water to 50 ml volumetric flask. Add enough sodium sulfate to saturate the water. Add 1 ml of 1:1 H_2SO_4 and exactly 5 ml of diethyl ether to the flask. Make to volume with saturated sodium sulfate solution and shake the contents vigorously for 1 minute (CAUTION: Ether pressure may cause loss of sample).

C Transfer 2 ml of the ether layer to a 10 ml volumetric flask. Evaporate the ether layer to dryness with air.

IV METHYLATION

A Pipette 1 ml of the BF_3-, methanol solution into each of the sample and standards flasks. Hold the flasks in a boiling water bath for 2 minutes with frequent swirling and then cool.

B Pipette exactly 1 ml of hexane into each flask. Make to volume with 2% Na_2SO_4 solution and shake the contents vigorously for one minute. Allow to stand for at least an hour before proceeding with the determinative technique.

V DETERMINATIVE STEP

A Inject 5 µl of each standard into a gas chromatograph equipped with an electron capture detector. Measure peak heights and prepare a standard curve.

B Inject 5 µl of the sample into the gas chromatograph. Measure the peak height and calculate the amount of silvex in the sample.

$$\frac{\text{NG silvex}/5\ \mu\text{l injection}}{10^{-6}} \times 200 \times 2.5 \times 40 = \text{mg/l silvex}$$

VI NOTES ON PROCEDURE

A This is a screening method for silvex which should also be applicable to other phenylalkanoic acid herbicides. For more sensitive and comprehensive methods consult reference 1.

B This method measures both silvex and its esters. A bicarbonate extraction technique for separating the acid and its esters in the original sample and measuring them independently has been applied (see reference 3).

REFERENCES

1 Burchfield, H. P. and Johnson, D. E. Manual of Methods for the Analysis of Pesticide Residues. Section IV (Extraction and Cleanup of Anionic Pesticides). DHEW, PHS, October 1964.

2 Gutenmann, Walter H. and Lisk, Donald J. Gas Chromatographic Method for Silvex. JAWWA 56:189 (1964).

3 Organic Pesticides in the Environment. Advances in Chemistry Series (#60). Determination of Silvex and its Low Volatile Esters in Water and Muds (John D. Pope, Jr., W.S. Cox, III, and A.R. Grzenda). Washington, D.C. American Chemical Society. (1966).

DATA SHEET

	NG Silvex/ 5 μl Injection	Peak Height
Standard	1.0 NG	
Standard	2.5	
Standard	5.0	
Sample		

THE BECKMAN PROCESS CARBONACEOUS ANALYZER

I BACKGROUND

Two approaches are generally used in evaluating the degree of organic water pollution:

1. Determination of oxygen (or other oxidants) required to react with organic impurities.
2. Measurement of organic carbon present in these impurities.

The first approach is represented by conventional BOD and COD tests.

The second approach in the past has been to oxidize the carbon to CO_2 which is collected and either weighed, titrated or measured manometrically.

All of these methods take considerable time (two hours to five days) and are subject to various degrees of inaccuracy. Although the BOD or COD become accepted technical and legal standards for measurement of organic pollution, they are historical and are essentially valueless in providing data for immediate determination of pollution sources or data for taking effective corrective action to control treatment processes.

II DESCRIPTION OF INSTRUMENTATION SYSTEM

The Beckman Process Carbonaceous Analyzer (PCA) is an automated "on-stream" version of the Laboratory Carbonaceous Analyzer employing the combustion/infrared method for determination of carbon in water developed by the Dow Chemical Co.(1) This technique provides an analysis of carbon in water samples in two minutes.

The Process Carbonaceous Analyzer includes an automatic sample injection valve, carrier gas system, combustion furnace, CO_2 sensitized non-dispersive infrared analyzer, and strip chart recorder all mounted, piped, and wired on a free-standing steel panel (72" × 30" × 28" HWD) (Slides #1 and #2).

III PRINCIPLE OF OPERATION

A Block Diagram

A simplified block diagram of the Process Carbonaceous Analyzer is shown in Slide #3.

B Operation

A water sample continuously flows through a 20 microliter hole in the slider insert of a sandwiched valve.

When triggered by a timer, the valve operates to transfer the sample plug into a flowing oxygen stream.

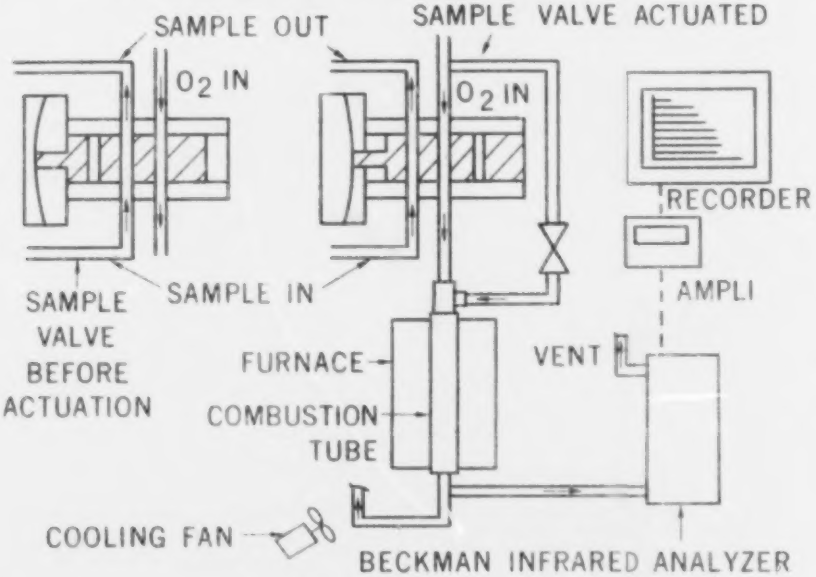
The oxygen injects the sample droplet into a catalytic combustion tube thermostated at 950°C in an electric furnace. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2).

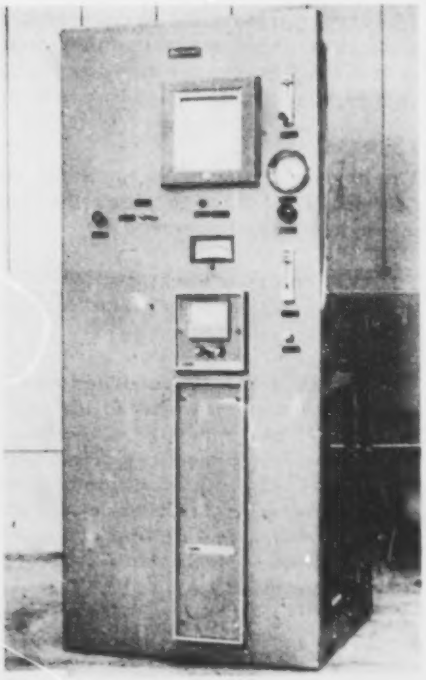
The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and removed.

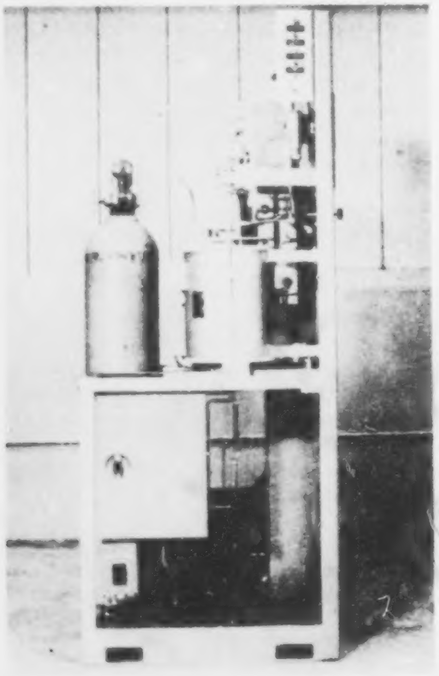
The CO_2 enters a Beckman Non-Dispersive Infrared Analyzer sensitized to measure CO_2.

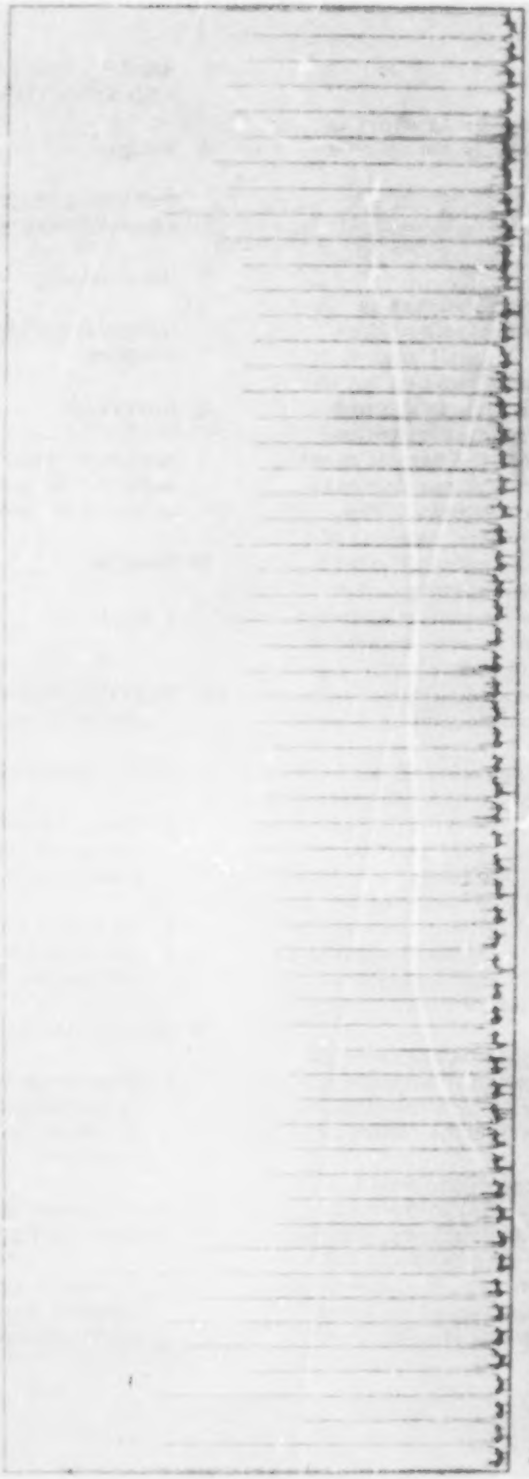
The transient CO_2 is recorded on a strip chart recorder as a peak (Slide #4). The peak height is a measure of the CO_2 present. The CO_2 present is directly proportional to the concentration of carbonaceous material in the original sample. By using standard solutions the chart can be calibrated in milligrams carbon per liter of sample.

Process Carbonaceous Analyzer Diagram


SAMPLE OUT
SAMPLE VALVE ACTUATED
O_2 IN
O_2 IN
RECORDER
AMPLI
SAMPLE IN
SAMPLE VALVE BEFORE ACTUATION
FURNACE
VENT
COMBUSTION TUBE
COOLING FAN
BECKMAN INFRARED ANALYZER







Typical Carbonaceous Analyzer chart readout showing the individual peaks of each analysis in a continuing series of analyses.

C Analysis Time

Total Analysis Time can be as short as two minutes but is typically set up on a five minute cycle.

IV APPLICATIONS

The Process Carbonaceous Analyzer is essentially applied to the analysis of dissolved carbon in water. A small amount of fine suspended solids can be handled but the exact degree has not been clearly defined and varies from application to application. Essentially we can say that the sample must be filtered. Sample filtration can decrease the "analyzed" carbon in a sample. The decrease would be due to filter removal of suspended carbonaceous matter. It can be rationalized that suspended carbonaceous pollution is obviously visible and that from a pollution control and enforcement standpoint, there should normally be no excuse for significant amounts of suspended carbonaceous pollution in an effluent to a receiving water.

While procedures have been worked out in the laboratory to eliminate or reduce the interference from inorganic carbon(2) this has not yet been successfully done with the Process Carbonaceous Analyzer. However, inorganic carbon interference may be accounted for if the inorganic carbon is relatively constant or it can be disregarded if the ratio of organic to inorganic carbon is sufficiently high for the analytical accuracy desired.

In other cases, the analyzer would serve as an alarming preventive monitor with the differentiation between organic and inorganic carbon determined in the control laboratory.

Considerable work has been done (much of it by the FWPCA at Taft Center) to correlate carbon analysis - or more precisely, Total Organic Carbon (TOC) - with BOD and COD. While potential discrepancies are immediately obvious, reasonable relationships on given wastewaters have been demonstrated.(3, 4)

V RANGE, REPEATABILITY, ACCURACY AND SENSITIVITY OF MEASUREMENTS

A Range:

Full scale range of the Analyzer can be adjusted from 0 to 50 to 0 to 4000 mg/l.

B Repeatability:

Normally ±2% of full scale on standard samples.

C Accuracy:

Analyzer reports 99+% of carbon in the sample. No compound has been found to which the method is not applicable.

D Sensitivity:

1 mg/l.

VI SYSTEM FOR DATA RECORDING OR TRANSMITTAL

A Local Recording (Analog)

1 Data is normally presented in peak (bargraph) format on 7 inch Strip Chart Recorder.

2 By use of an optional electronic peak memory device recording can be in continuous "trend" format.

B Remote Recording (Analog)

1 Slave peak recorder can be located up to approximately 2000 feet distance by use of optional "retransmitting slidewire" in local recorder.

C Data Transmittal and Remote Recording (Analog or Digital)

1 By use of an optional electronic peak memory device, analog data can be made continuously available for access

CARBON-NITROGEN-HYDROGEN ANALYZER

I INTRODUCTION

Over the years, the classical Pregl and Dumas procedures[3, 5] for the microdetermination of carbon, hydrogen, and nitrogen provided the microchemist with reliable analytical data. However, the disadvantages of these traditional methods:

- TIME-CONSUMING PROCEDURE - about 45 minutes is required for a complete carbon, hydrogen, nitrogen analysis.
- SPECIAL LABORATORY FACILITIES - for reliable microanalytical results, a filtered air atmosphere, in a vibration-free, temperature and humidity controlled environment is required.
- SPECIAL PRECISION EQUIPMENT - measurement of small, but real, differences in the weight of gas absorption tubes requires a high sensitivity, high capacity microbalance and a skilled operator.
- HIGHLY SKILLED TECHNICIANS - because of the delicate and tedious operations involved, unusually competent technicians are essential.
- HIGH COST/ANALYSIS - due to above factors.

were also well known to the microchemist. Recognizing that the high cost/analysis was due to the expense involved in creating the necessary physical environment, and training competent technicians as well as the time lost by the researcher waiting for results, various instrumental methods for automating the Pregl and Dumas procedures were developed.

The acceptability of these innovations to the microchemist was determined, for the most part, by the recognized analytical limitations, or allowable errors, of the analyses. As Dr. A. Steyermark[6] pointed out, "...the determination of carbon and hydrogen is always in order but is of no value if one is trying to differentiate between two compounds having these values so close together that they fall within the limits of allowable error. For example, suppose two such compounds have theoretical values of the following:

(a)	Compound I	Compound II
	C = 66.23%	C = 65.73%
	H = 4.76%	H = 4.36%

If a carbon-hydrogen determination gave

C = 66.03%   H = 4.56%

it would be impossible from this to say which of the two compounds, I or II, had been analyzed since the allowable error on both carbon and hydrogen are ± 0.3%." Obviously, with an allowable error of ± 0.3%, experimentally determined carbon values cannot be used to identify adjacent members of a homologous series of organic compounds.

In practice, the allowable error of ± 0.3% is accepted as the criterion for evaluating the reliability of duplicate test results and the analytical methods by which such values are obtained. However, the accepted 0.3% difference between duplicates, which varies somewhat from laboratory-to-laboratory or method-to-method, may not be the best criterion for evaluating reliability, or for comparing data from different sources. This concept is discussed more fully in the Data Evaluation Section.

In the late 1950's two instruments designed to overcome some of the problems involved in the microdetermination of carbon, hydrogen and nitrogen were placed on the market. To determine all three elements two instruments were required; one for carbon and hydrogen, and the other for nitrogen. While both instruments were designed for the automation of the basic Pregl and Dumas methods, only the sample combustion step was automated. But, the tedious and delicate weighing of the sample and the relatively heavy carbon dioxide and water absorption tubes, before and after combustion, was still a manual operation requiring a skilled technician. As with the carbon and hydrogen analyzer, the combustion step in the Dumas method was automated, but the sample weighing operation was still a manual one. However, a specialized nitrometer for measuring the gas volume directly in cubic centimeters was included in the nitrogen analyzer. With this procedure, nitrogen analyses could be performed in 10 minutes.

However, with today's rapid technological advances, a simpler and more fully automated method for the microdetermination of carbon, hydrogen and nitrogen was necessary to meet the demand for more efficient laboratory operations. Several investigators[2] described instrumental methods to measure the products of combustion, and gas chromatographic techniques[1, 4, 7, 8] were also used extensively with good results. Later, commercial gas chromatographic instruments were placed on the market. In one of these, combustion takes place in a flowing stream of oxygen rather than in the recommended statis system and water must be converted to acetylene prior to injection into the single column of the chromatograph. The components are frozen-out and then injected in the chromatograph.

In a second instrument, a mixture of helium and oxygen is used as the carrier gas. After combustion is complete, oxygen is removed from the carrier gas by reaction with metallic copper while the combustion products (CO_2, H_2O and N_2) are selectively separated in the detection section of the instrument. The presence of gases other than carbon dioxide, nitrogen, and water in the effluent stream will be measured as either CO_2 or N_2, and result in high values for these constituents.

The F & M Model 180 C-H-N Analyzer, which involves a modified Dumas technique for combustion, helium as the carrier gas, and a dual-column, dual-detection system, was designed to provide optimum sample-oxidant contact for combustion under static conditions, thus eliminating certain operational problems inherent in the other commercially available C, H and N instruments. The simplicity of operation, plus elimination of multiple weighings on a high-capacity microbalance enables a laboratory technician to obtain reliable results in a minimum of time.

II BASIC COMBUSTION CHEMISTRY

Regardless of the method used to determine carbon, hydrogen and nitrogen in an organic material, the chemistry involved is basically the same. The sample is decomposed by heat, and the products are oxidized -- carbon is converted to carbon dioxide, hydrogen to water and combined nitrogen to the free gas -- as shown in the following general reactions:

$$\begin{matrix}\text{Organic}\\ \text{Sample}\end{matrix} \xrightarrow[\text{Inert Atm.}]{\Delta + \text{ cat.}} \begin{matrix}\text{Decomposition}\\ \text{Products}\end{matrix}$$

$$\begin{matrix}\text{Decomposition}\\ \text{Products}\end{matrix} \xrightarrow[\text{Inert Atm.}]{\Delta + \text{ cat.}} CO_2 + H_2O + N_2$$

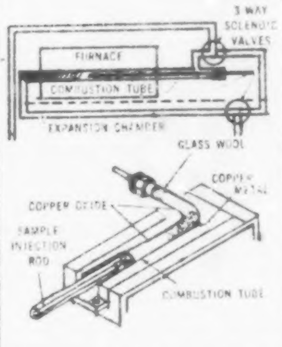
The optimum combustion conditions, established by Professor W. Simon[9] require adequate temperatures, an oxygen atmosphere, a suitable oxidation catalyst and a static system. These are recognized as critical factors in the design of instrumental systems for carbon, hydrogen, and nitrogen analyses. In recently developed commercially available instruments, oxygen, or an oxygen (3%) - helium mixture, is used as the carrier gas. The latter systems also provide for static conditions during combustion, whereas, with oxygen alone, a continuous gas flow is maintained. Since large volumes of carrier gas are required when the oxygen is limited to that in the oxygen (3%) - helium mixture, a system in which sufficient oxygen is produced under static conditions during combustion would simplify the mechanics and improve the efficiency of this procedural step. Such a system is an integral part of the recently developed Model 180 Analyzer.

In the Model 180 instrument, the sample elements are converted to the desired oxidation state (CO_2, H_2O and N_2) by means of a Ag_2O-MnO_2 catalyst-oxidant under static conditions. The oxidation reaction takes place in the combustion zone of the furnace section; see Figure 1.

Figure 1

SAMPLE INJECTION & COMBUSTION SYSTEMS

F&M MODEL 180 ANALYZER



The general reactions in the furnace (700-1000° C) are as follows:

$$Ag_2O + MnO_2 \xrightarrow{\Delta} 2\,Ag + MnO + O_2$$

$$\text{Sample} + O_2 \xrightarrow{\Delta} CO_2 + H_2O + N_2$$

An oxygen atmosphere in the combustion area is produced by decomposition of the silver oxide in the oxidant:

$$2\,Ag_2O \xrightarrow{300^\circ C} 4\,Ag + O_2$$

Then as the decomposition temperature of MnO_2 is reached, oxygen is also released in the immediate vicinity of any non-volatile pyrolytic compounds thus ensuring complete oxidation of the sample decomposition products.

$$2\,MnO_2 \xrightarrow{500^\circ C} 2\,MnO + O_2$$

The various oxides of carbon and/or nitrogen are converted to carbon dioxide and nitrogen by reaction with the CuO-Cu packing in the modified Dumas combustion tube.

$$CuO + CO \xrightarrow{\Delta} CO_2 + Cu$$

$$Cu + \text{Nitrogen Oxides} \xrightarrow{\Delta} CuO + N_2$$

Excess oxygen in the combustion tube is removed by the hot reduced copper before the effluent gases are admitted to the chromatographic section.

$$2\,Cu + O_2 \xrightarrow{\Delta} 2\,CuO$$

After carbon, hydrogen and nitrogen are converted to the desired oxidation state by combustion, the quantity of each can be determined by gas chromatography or by some other method. Regardless of the method selected for making the final measurements, the chemistry involved up to that point is essentially the same.

III DESIGN CONCEPT

Basically, the F & M Model 180 Carbon, Hydrogen and Nitrogen Analyzer was designed to overcome the obvious disadvantages of the classical Pregl and Dumas methods. This necessitated the development of systems to:

- Replace the multiple weighing manipulations which require a high-capacity, environment-sensitive microbalance and highly skilled technicians.

- Decrease the time required for a complete carbon, hydrogen and nitrogen sample analysis.

- Permit the simultaneous determination of carbon, hydrogen and nitrogen in a single sample.

- Simplify the computations.

The first of the design objectives was achieved by replacing the conventional microbalance with a low-capacity, environment-insensitive electro-microbalance and eliminating the heavy adsorption tubes. Also, all operations are performed under normal laboratory conditions thus eliminating the need for the special laboratory environment required for conventional microanalysis.

In order to decrease the time required for a conventional microanalysis and to permit the simultaneous determination of carbon, hydrogen and nitrogen in a single test portion, a modified Dumas combustion technique and a dual-column, dual-detector gas chromatograph were combined to give a semi-automatic system. The instrument consists of three sections:

- An injection system for introducing the sample into the hot zone of the furnace.

- A furnace section where the sample is oxidized and the reaction products are stored prior to injection into the chromatographic columns.

- A chromatograph section where the reaction products are separated into individual components and measured.

As the individual components pass through the dual-column, dual-detector portion of the chromatograph, they are separated into bands which appear as peaks on the recorder chart, see Figure 2. The concentration of each component is then calculated from peak-height measurements; under certain conditions, calculations may be based on peak-area measurements. The time required for a complete carbon, hydrogen and nitrogen sample analysis is about 10 minutes as compared to 45-80 minutes by conventional microanalytical methods.

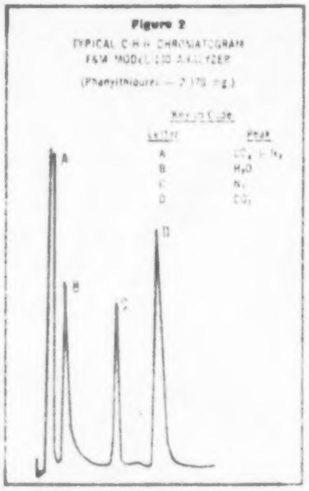


Figure 2
TYPICAL C-H-N CHROMATOGRAM
F&M MODEL 180 ANALYZER
(Phenylthiourea — 2.175 mg.)

In the design of the M-180, a Cahn Ratio Electrobalance® is combined with a Ratio-Recording System to electrically compensate for the variation in recorder response due to changes in sample weight. Since this combination in effect divides the recorder signal by the sample weight, the peak height for a given compound then becomes a constant. The advantages of this Electrobalance-Recorder combination include:

1 Convenience in checking instrument performance.

2 Elimination of errors in reading and recording sample weights.

3 Simplification of computations.

The mechanism involved in electrically compensating for the recorder response to sample weight is as follows: (1) a voltage, proportional to sample weight, is applied across the slidewire of the recorder as the sample is weighed on the Electrobalance; (2) recorder sensitivity which varies inversely with sample weight automatically adjusts to compensate for the input voltage from the Electrobalance;

(3) the compensated recorder signal thus becomes a function of sample composition and is independent of sample weight.

The reproducibility of peak height measurements with the Ratio-Recording System in operation is shown in Figure 3 for four samples, while the precision of the carbon, hydrogen, nitrogen values, derived from the chromatograms, are presented in Table 1.

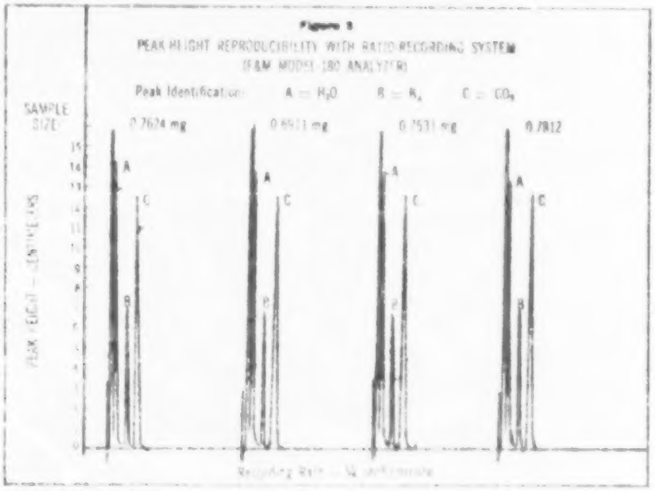


Figure 3

PEAK HEIGHT REPRODUCIBILITY WITH RATIO-RECORDING SYSTEM (F&M MODEL 180 ANALYZER)

TABLE 1

Reproducibility of Peak-Height Measurements With Ratio-Recording System (F & M M-180 C-H-N Analyzer)

Samples	Sample Weight (mg)	Peak-Height Measurements Carbon	Hydrogen	Nitrogen
		(Centimeters)		
1	0.7624	12.60	14.05	6.62
2	.6911	12.59	13.36	6.65
3	.7531	12.58	13.78	6.51
4	.7812	12.60	13.25	6.65
Average - $\bar{X}$		12.59	13.61	6.60
Range - R		0.02	0.80	0.14
Std. Deviation - S		01	.12	.07
Conversion Factor K*		4.11	372	3.06

*$\overline{KX}$ = C, H, N Values in Percent

Calibration curves for both peak height and peak area are prepared by processing appropriate-size weighed portions of a standard sample (a material of known composition), and plotting the peak-heights or peak-areas recorded for the reaction products, CO_2, H_2O and N_2 against the corresponding known values for carbon, hydrogen and nitrogen. Typical peak-height and peak-area calibration curves are shown for carbon in Figure 4.

At higher carbon values, (see Figure 4), the peak-height calibration curve is no longer linear. Furthermore, the Ratio Recording System cannot be used with larger sample sizes; above 0.8 milligrams, the detection system is overloaded to such an extent that the relationship between peak-height and component concentration is no longer linear. In these cases, the Ratio Recording System is cut out of the recorder circuit and test results are calculated from specially prepared peak-height calibration curves or from peak-area measurements; under these circumstances, sample weights must be recorded. Obviously, the computational convenience associated with the use of the Ratio Recording System and peak-height measurements more than compensates for any inconvenience involved in adjusting the sample size to the optimum weight.

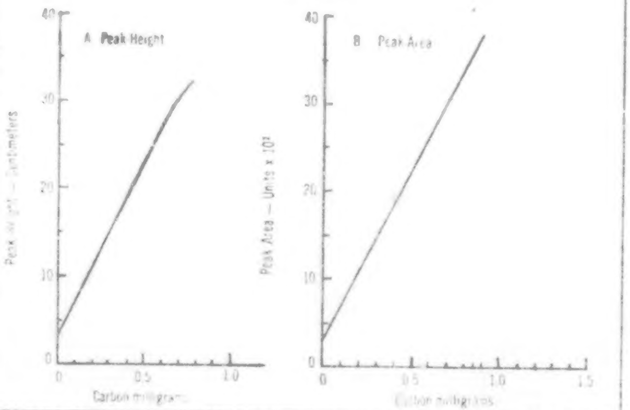
With the F & M Model 180 Analyzer a simultaneous determination of carbon, hydrogen and nitrogen in the sample weight range of 0.5 to 1 mg can be completed within 10 minutes; with the Ratio Recorder disconnected, samples as small as 0.25 mg can be analyzed. Only a single sample is required for the complete analysis and all analyses are made under normal laboratory conditions. Consequently, the obvious disadvantages of the classical Pregl and Dumas methods are eliminated.

IV OPERATIONAL FEATURES

In the Model 180 Analyzer, the bypass carrier gas system and expansion chamber permit variation in the time required for complete oxidation of the sample and also provide partial cooling and storage of the reaction product. The combustion temperature, ambient to 1000° C, is controlled with an F & M Variable Power Regulator (VPR) and the combustion tube is packed with cupric oxide and reduced copper wire to ensure complete oxidation and reduction. With this combustion system, most samples are completely oxidized in 2 minutes at 800° C.

Figure 4

PEAK-HEIGHT AND PEAK-AREA CALIBRATION CURVES
F&M MODEL 180 ANALYZER

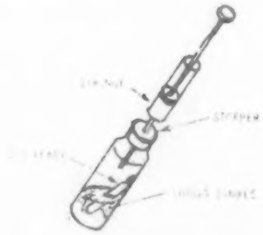


The injection system, through which the sample is placed in the combustion area, was designed to minimize dead volume and to maintain a gas tight seal during sample processing. Because of the sample-injection and combustion-system design, operating temperatures can be maintained on a continuous basis. Thus, the need for a periodic cooling cycle is eliminated.

Solid samples are weighed on an Electrobalance in preformed, preconditioned (washed in organic solvent and heated at 200-500° C for 12 to 18 hours) aluminum boats which are made from one-mil aluminum foil. Prior to injection into the combustion system, the sample in the boat is covered with a layer of catalyzed oxidant to provide contact oxidation conditions in the combustion area.

Liquid samples, contained in thin-wall aluminum capillary tubes, are introduced into the M-180 C-H-N Analyzer in the same manner as solids. Prior to injection into the Analyzer, an empty pre-weighed capillary tube is placed in a small vial of the liquid sample (see Figure 5) and filled by creating a partial vacuum in the tightly sealed container. The loaded capillary is then sealed and reweighed to obtain the sample weight. While the liquid sample loading and weighing procedure is not as simple as that for handling solids, reliable carbon, hydrogen and nitrogen values are obtainable using this sampling technique.

Figure 5
LIQUID SAMPLING TECHNIQUE
(M-180 C-H-N ANALYZER)



Since an inert carrier gas, such as helium, is necessary to achieve the desired detection sensitivity, a separate oxidant with the following characteristics is required:

- Must be sufficiently powerful to oxidize the sample completely.
- Oxidation must be rapid and quantitative.
- The catalyst must be pure and free from CO_2, N_2 or H_2O contamination.

For these reasons, Ag_2O--MnO_2 was selected as the oxidant in the C-H-N procedure.

After combustion is complete, water in the reaction gases is separated from carbon dioxide and nitrogen in Column 1, which is packed with Carbowax 1500 on Haloport F* support, and then measured (sensed) by Thermal Conductivity Detector I. The water is then removed from the gas stream by a Dow Dessicant 812^{R} drier at the head of a hold-up column which is provided to delay the elution of carbon dioxide and nitrogen until the water peak is fully developed.

Nitrogen is separated from carbon dioxide in Column II, which is packed with granulated Pittsburgh Coke and Chemical BPL activated carbon*. Then, each gas is measured as it passes through Thermal Conductivity Detector II. The signals developed by the Thermal Conductivity detectors, as the gases pass through the columns, are recorded on a Brown Instrument Recorder.

The entire chromatographic system is operated isothermally by housing the components in an insulated oven which is maintained at a constant temperature of 100-150° C. Cartridge heaters and a thermoswitch embedded in a one-inch aluminum plate, which extends over the entire oven base, maintain the oven temperature at the desired level.

---

*Available from F & M Scientific Corporation, Avondale, Pennsylvania.

V DATA EVALUATION

In microanalytical work, an allowable error of ± 0.3%[6] is the accepted reliability criterion for carbon, hydrogen and nitrogen analyses. As applied by the microchemist, duplicate test results for any of the three constituents are considered acceptable if the difference (D) does not exceed this allowable error. Consequently, the accepted or allowable error is a recognized measure of the precision or reproducibility of values obtained by a given procedure and can be used as a comparison criterion for evaluating different methods of analysis. In other words, the allowable error of ± 0.3% defines the limits within which differences in duplicates are expected to lie and thus is considered as the accepted limit of analytical error for carbon, hydrogen and nitrogen analyses.

However, the limit of error for these analyses can also be derived from the standard deviation (S) which is a generally recognized term for expressing precision or reliability. The formula for limit of error (L. E.) is as follows:

$$\text{L.E.} = \pm KS$$

where

S = Standard deviation

K = Probability level factor based on the area under the Normal Distribution curve. For practical purposes,

$K = 1$ = 67% probability level, or the area under the curve designated as $\bar{X} \pm S$.

$K = 2$ = 95% probability level, or the area under the curve designated as $\bar{X} \pm 2S$.

$K = 3$ = 99% probability level, or the area under the curve designated as $\bar{X} \pm 3S$.

$K = 4$ = 100% probability level, or the area under the curve designated as $\bar{X} \pm 4S$.

Now, the relationship[10, 11] between standard deviations (S), and the acceptable difference (D) or range (R) in duplicates, is as follows:

$$S = (R_f)$$

when $R = D$,

$$S = D\,(R_f)$$

where:

S = Standard deviation

R = Range or difference between high and low values in a set of data

D = Difference between duplicates

R_f = Conversion factor for duplicates = 0.885

So, if

D = Allowable Error...and the Allowable Error = ± 0.3%

then,

$$D = 0.3\%$$

and

$$S = 0.30 \times 0.885 = 0.27\%$$

Thus, the limit of error, depending on the probability level selected, becomes

$$\begin{aligned}\text{L.E.} &= \pm 0.27\% \\ &= \pm 0.54\% \\ &= \pm 0.81\% \\ &= \pm 1.08\%\end{aligned}$$

Obviously, the limit of error (based on the standard deviation) at a 67% probability level is equivalent to the accepted limit of analytical error for carbon, hydrogen, and nitrogen analyses. Thus, only two-thirds of the difference between duplicates can be expected to lie within the accepted limits of analytical error. Therefore, an evaluation criterion based on the limit at a 95% probability level is recommended.

The data summarized in Table 2 for laboratories 1 thru 6 were derived from duplicate Pregl values published in the Journal of the American Chemical Society for a one-year period. While the average difference between duplicates varies somewhat from laboratory to laboratory, the overall difference between duplicates (0.24) is within the limit of error at a 95% probability level for both carbon and hydrogen. Furthermore, evaluation of F & M Model 180 data, see Table 2, yielded a comparable overall difference between duplicates. Therefore, test results of comparable precision, or reliability, are obtainable by the Pregl and the F & M Model 180 Analyzer.

TABLE 2

Reproducibility[a] of F & M M-180 & Pregl Data

		Carbon Data			Hydrogen Data		
Method	Laboratory	Number of Duplicates (N)	Average Difference (D)	Standard Deviation (S)[c]	Number of Duplicates (N)	Average Difference (D)	Standard Deviation (S)[c]
M-180	A	44	0.27	0.24	44	0.10	0.09
	B	20	.19	.17	20	.13	.11
	Over-All	64	0.23	0.20	64	0.12	0.11
Pregl[b]	1	3	0.22	0.19	3	0.11	0.10
	2	10	.15	.13	10	.09	.08
	3	9	.33	.29	9	.15	.13
	4	6	.35	.31	6	.15	.13
	5	11	.27	.24	11	.11	.10
	6	4	.10	.08	4	.10	.09
	Over-All	43	0.24	0.21	43	0.12	0.11

a Based on duplicate determinations

b Published data

c $S = DR_f$ where $R_f = 0.885$ (Based on N = 2 for duplicate determinations)

VI APPLICATION DEVELOPMENT

Since the development of the Model 180 C-H-N Analyzer, this method has been used in both the F & M and customer laboratories to analyze a variety of organic materials. Operational-type carbon, hydrogen and nitrogen calibration curves, such as those used in these analyses, are shown in Figure 6 while an operational set of peak-height calibration data is given in Table 3. The excellent reproducibility of the system blank and calibration factors is indicative of the reliability of the carbon, hydrogen and nitrogen test results.

TABLE 3

C-H-N Peak Height Calibration Data
(F & M M-180 C-H-N Analyzer)

Standard: Cyclohexanone 2, 4 Dinitrophenylhydrazine
Composition: C = 51.79% H = 5.07% N = 20.16%

	Calibration Data*					
	Carbon		Hydrogen		Nitrogen	
Run No.	Peak Height (cm)	Factor (% C/cm) K_c	Peak Height (cm)	Factor (% H/cm) K_H	Peak Height (cm)	Factor (% N/cm) K_N
1	11.98	4.323	6.02	0.843	3.03	6.53
2	11.94	4.337	6.14	.827	3.08	6.53
3	11.98	4.323	6.20	.818	3.35	6.01
4	11.99	4.320	6.01	.843	3.08	6.54
5	11.98	4.323	6.00	.844	3.10	6.51
6	11.99	4.320	6.02	.843	3.09	6.52
7	11.94	4.337	5.96	.847	3.00	6.72
8	11.96	4.330	6.01	.845	3.02	6.67
9	11.94	4.337	5.90	.859	3.02	6.67
10	11.96	4.330	5.96	.850	3.05	6.62
11	11.96	4.330	6.09	.832	3.07	6.32
No. Measurements(N)		11		11		11
Average $\overline{X}$		4.328		0.841		6.51
Std. Deviation (S)		0.067		.011		0.41

*Blank = 2.00 for C, H_2 & N_2; Attenuation = 32.

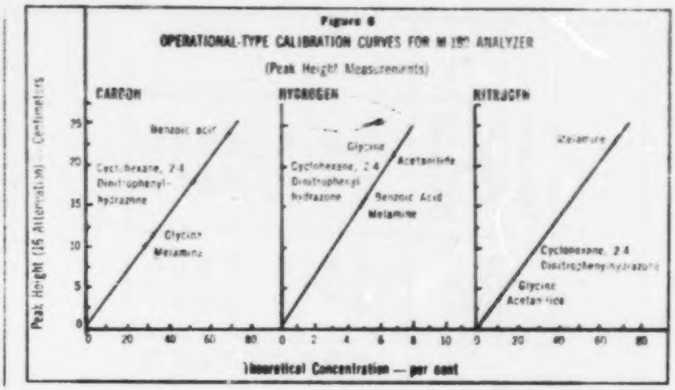


Figure 6

OPERATIONAL-TYPE CALIBRATION CURVES FOR M 185 ANALYZER

(Peak Height Measurements)

After standard calibration curves are initially established with compounds of known composition, component factors (K) are derived in the following manner and are used for calculation purposes:

Carbon K_c =

$$\frac{\text{Percent Carbon in Standard}}{\text{Peak Height } CO_2 \text{ Peak--}CO_2 \text{ Blank}} = \%\ C/\text{cm.}$$

Hydrogen K_H =

$$\frac{\text{Percent Hydrogen in Standard}}{\text{Peak Height } H_2O \text{ Peak--}H_2O \text{ Blank}} = \%\ H/\text{cm.}$$

Nitrogen K_N =

$$\frac{\text{Percent Nitrogen in Standard}}{\text{Peak Height N--N Blank}} = \%\ N/\text{cm.}$$

The reproducibility of the blank measurements and calibration factors from day-to-day is a convenient method for evaluating operator and instrument performance.

The type of results obtainable when peak-area measurements are used is shown in Table 4 while similar data based on peak-height measurements are presented in Table 5. From a comparison of the data in these two tables, it was concluded that:

- Computations based on peak-area and peak-height measurements yield equally valid test results.
- Sulfur and halogens in the sample apparently do not interfere with the analysis.
- No special sample handling is needed for a variety of sample types and different bondings.
- Low concentrations of carbon in combustible materials can be determined readily and without interference.

TABLE 4
C-H-N Peak-Area Data
(F & M M-180 C-H-N ANALYZER)

Sample Identification	Carbon			Hydrogen			Nitrogen		
	Known (Y)	Found* X (Percent)	S	Known (Y)	Found* X (Percent)	S	Known (Y)	Found* X (Percent)	S
Camphor	78.80	78.60	---	10.53	10.27	---	---	---	---
Polyacrylonitrile	68.00	67.90	0.15	5.70	5.70	0.16	26.90	26.50	0.10
5-Hydoxyindole Acetate[a]	63.30	63.40	.31	8.07	8.64	.08	8.42	8.36	.18
Homovanillic Acid	59.40	59.60	.34	5.49	5.39	.08	---	---	---
Glutamic Acid	40.80	40.80	.41	6.12	6.22	.27	9.56	9.37	.19
		40.80	.42		6.17	.23		---	---
Histidene-HCl·H_2O	34.44	34.90	.19	5.27	5.56	.26	20.10	19.60	.21
Arginine-HCl	34.20	34.10	.58	7.13	7.24	.17	26.55	26.10	.14
Glycine	31.99	32.60	.15	6.68	6.75	.06	18.67	18.30	.26
Cystine	29.69	30.50	.24	5.07	5.16	.15	11.78	11.60	---

NOTE: Data tabulated in descending order of carbon values.

* $\bar{X}$ = average; S= std. deviation; N = 10 or more measurements

[a] Cyclohexyl ammonium salt.

TABLE 5
C-H-N Peak-Height Data
(F & M M-180 C-H-N ANALYZER)

Sample Identification	Carbon			Hydrogen			Nitrogen		
	Known Y	Found* X (Percent)	S	Known Y	Found* X (Percent)	S	Known Y	Found* X (Percent)	S
Naphthalene	93.74	93.75	0.21	6.29	6.60	0.06	---	---	---
Cholesterol	83.87	83.81	.27	11.99	11.98	.23	---	---	---
Benzoic Acid	68.84	68.85	.18	4.95	4.86	.16	---	---	---
Triphenylphosphate	66.26	66.54	.22	4.63	4.69	.05	---	---	---
p-fluorobenzoic Acid	60.01	59.88	.19	3.60	3.80	.04	---	---	---
Phenylthiourea	55.19	55.26	.16	5.29	5.32	.14	---	---	---
S-Benzylthiouronium[a]	47.36	47.45	.22	5.47	5.51	.42	18.41	18.20	0.2
Hexachlorobenzene	25.30	25.20	.51	---	---	---	13.60	14.00	0.3
Sulfur	0.11	0.11	---	---	---	---	---	---	---
	.31	.27	---	---	---	---	---	---	---
	.85	.85	---	---	---	---	---	---	---

NOTE: Data tabulated in descending order of carbon values.

*Average ($\bar{X}$) and standard deviation (S) based on 10 or more measurements (i.e., N = 10).

[a] Chloride salt.

Because of the convenience and simplicity of the peak-height method for computing test values, a variety of organic materials were analyzed using the M-180 procedure and results were calculated on the basis of peak-height measurements; these data are tabulated in Table 6.

Since these samples present varying degrees of difficulty when analyzed for carbon and hydrogen by classical methods, the agreement between the theoretical content and that found by the M-180 procedure is considered very satisfactory. This work confirms the reliability of the F & M Model 180 C-H-N Analyzer.

By changing the carrier gas system to hydrogen and the combustion tube packing to a hydrogenation catalyst, the oxygen content of reducible metal oxides can probably be determined with the F & M Model 180 Analyzer.

TABLE 6
C-H-N Data for Variety of Organics
(F & M M-180 C-H-N ANALYZER)

Sample Identification[b]	Carbon		Hydrogen		Nitrogen	
	Theor.	Found[a]	Theor.	Found[a]	Theor.	Found[a]
	(Percent)		(Percent)		(Percent)	
Anthracene	94.34	94.36	5.66	5.99	---	---
Hexane[c]	84.60	84.75	16.38	16.80	---	---
Octane[c]	84.12	83.90	15.89	16.05	---	---
Triethylamine[c]	71.21	71.60	14.94	14.96	---	---
Unknown 2	69.13	69.05	11.35	11.54	3.51	3.15
Unknown 6	66.65	66.90	4.19	4.19	29.15	29.40
Triphenyl phosphate	66.26	66.57	4.63	4.69	---	---
Unknown 8	63.95	63.90	5.60	5.92	2.98	2.35
Unknown 9	62.90	62.80	2.84	2.72	11.30	10.65
Acetone[c]	62.04	62.45	10.41	11.05	---	---
p-fluorobenzoic acid	60.01	59.88	3.60	3.79	---	---
Acetonitrile[c]	58.51	58.70	7.37	7.34	---	---
Unknown 7	57.80	57.55	7.52	7.62	7.46	7.43
Unknown 10	59.69	59.39	5.73	5.64	4.97	4.55
Unknown 1	52.17	52.30	5.73	5.94	4.68	4.50
Potassium acid phthalate	47.05	47.10	2.47	2.69	---	---
Sulfanilamide	41.85	42.04	4.68	4.38	---	---
Unknown 4	37.93	37.75	2.65	2.80	22.10	21.80
2, 4-dinitrophenylhydrazine	36.37	36.54	3.05	2.89	---	---
Unknown 3	34.48	34.72	6.94	7.02	40.21	39.75
O-iodobenzoic acid	33.90	33.79	2.03	1.96	---	---
Unknown 5	33.39	33.30	1.68	1.59	15.56	15.35
Chloroform[c]	10.10	10.40	0.84	0.93	---	---

NOTE: Data tabulated in descending order based on carbon content.

[a] Average value based on 3 or more measurements.

[b] Unknown samples 1 thru 10 were unidentified organic materials submitted for analyses-theoretical values supplied at a later date.

[c] Liquid samples-average based on duplicate measurements.

In this method, the oxygen content of the sample would be calculated from the water peak-height measurement. However, in order to determine the oxygen content of organic materials, modification of the M-180 would be necessary.

VII SUMMARY

The classical Pregl and Dumas methods for the microdetermination of carbon, hydrogen and nitrogen in organic materials involve tedious, time-consuming microanalytical techniques which require expensive, special laboratory facilities and highly skilled technicians. But, reliable test results are obtainable with these traditional procedures. With the Model 180 Carbon, Hydrogen, and Nitrogen Analyzer, however, equally acceptable analyses can be made simultaneously in about 10 minutes under normal laboratory conditions.

With this modern instrumental method, samples ranging in size from 0.5-1.0 mg can be analyzed with the Ratio-Recording System in operation. If the total carbon is greater than 0.60 mg, however, either the Ratio-Recording System must be cut out of the circuit and special peak-height calibration curves prepared, or peak-area measurements must be used to compute test values. Furthermore, samples of less than 0.25 mg can be analyzed but the Ratio-Recording System must be disconnected during the analysis. The applicability of the Model 180 to the analysis of such small samples is a decided advantage when only small quantities of an unusual or rare material are available and only a limited amount can be provided for analysis.

The sample injection and combustion section of the M-180 was designed to ensure complete combustion in a closed system. Furthermore, the reaction gases can be retained in the expansion chamber of the system, if necessary, while the combustion products from the preceding samples are being separated and measured in the chromatograph section of the instrument; this permits the operator to process samples on a continuous basis.

The chromatographic section of the M-180 instrument provides a rapid, automatic method for separating and eluting each constituent and for measuring the amount of the individual sample components. This permits the simultaneous determination of carbon, hydrogen and nitrogen, without additional weighings or sample handling. Since this automated process replaces many of the tedious, time-consuming operations of the older conventional micromethods, the advantages inherent in the F & M Model 180 procedure are readily apparent.

Since the development of the F & M Model 180 Carbon, Hydrogen and Nitrogen Analyzer, problems such as the following have been handled successfully:

- Establishing empirical formula for organic compounds in chemical, petrochemical, biomedical, food, and agricultural laboratories.
- Concentration of carbon in spent catalyst.
- Boiler efficiency as a function of coal composition.
- Ratio of carbon to hydrogen in tar.
- Carbon content of refined sulfur.

Furthermore, the validity of the carbon, hydrogen, and nitrogen test results is not affected by sulfur or halogens in a sample.

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REFERENCES

1 Duswalt, Allen A. and Brandt, Warren W. Anal. Chem., 32, 272. 1960.

2 Haber, Herbert S. and Gardiner, Kenneth W., Microchem. J., VI No. 1, 83. 1962.

3 Niederl, J.B. and Niederl, V. Quantitative Organic Analysis, John Wiley and Sons, Inc., New York. 1942.

4 Nightingale, C.F. and Walker, J.M. Anal. Chem., 34, 1435. 1962.

5 Steyermark, A. Quantitative Organic Microanalyses, Blakeston, Philadelphia. 1951.

6 Steyermark, A. Quantitative Organic Microanalysis, 2nd Ed. P. 7. Academic Press, New York and London. 1961.

7 Sundberg, O.E. and Maresh, Charles. Anal. Chem., 32, 274. 1960.

8 Vogel, Alfred M. and Guattrone, Joseph J., Jr. Anal. Chem., 32, 1754. 1960.

9 Simon, W. Program Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Abstract No. 137, p. 73. 1963.

10 Snedicor, G.W. Statistical Methods, 4th Ed., The Collegiate Press, Inc., Ames, Iowa. 1946.

11 Dexon & Massey. Introduction to Statistical Analysis, 1st Ed., McGraw-Hill Book Company, New York. 1951.

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